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THE
JOURNAL OF INFECTIOUS DISEASES

The Journal of Infectious Diseases

Founded by the Memorial Institute for Infectious Diseases

EDITED BY

LUDVIG HEKTOEN AND EDWIN O. JORDAN

IN CONJUNCTION WITH

FRANK BILLINGS F. G. NOVY

W. T. SEDGWICK

Volume II

1905

Chicago, 1905

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The
Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. II *January 12, 1905* NO. 1

SOME PHYSICAL PROPERTIES OF ENZYMES.*

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CONTENTS.

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PANCREATIN: Its removal from solution by paper filtration; retention by the Berkefeld bougie and by the collodion filter; indialysability through collodion wall.

INTRODUCTION.

THE fact that enzyme solutions decrease in activity as the result of filtration has been frequently observed. Musculus[1] in 1874 called attention to the retention of the enzyme urase by filter paper, a fact which Lea[2] explained by the assumption that urase is not a soluble ferment. Miquel,[3] however, demonstrated the solubility of this enzyme.

*Received for publication August 4, 1904.

Wortmann[4] in 1890 attempted to demonstrate the presence of a ferment in green leaves. He tested the filtered aqueous extracts of crushed leaves for diastatic activity, and the leaves whose filtrates did not invert starch he declared free from diastase. This work elicited from Brown and Morris[5] (1893) the following:

> The absence of diastase from a clear filtrate obtained after merely digesting the crushed leaves with water for three or four hours is no sufficient proof that the leaf does not contain a starch-transforming enzyme: for it is well known to those who have spent much time in separating such enzymes from vegetable or animal tissues that the protoplasm often parts with its enzyme with difficulty, and that it is often almost impossible to obtain from such a preparation a clear filtrate which has any hydrolyzing action, although an energetic hydrolysis may be produced by contact with the tissue itself, or by the employment of a turbid filtrate containing the finely divided tissue in suspension.

They cite in evidence the following experiment:

> The leaf of Helianthus tuberosus was extracted by the prolonged action of water upon the crushed and pounded fresh leaf. The influence of micro-organisms was guarded against by the use of chloroform. The action of the filtered extract was then compared with a like maceration of the leaves themselves. The diastatic activity of 10 g. of the dried leaf of Helianthus, acting by contact, was found to be 3.78, while that of the filtered extract of 10 g. of the same leaf was 0.53.

They refer, further, to the observation of Brown and Herron[6] (1880) that the clear, filtered, aqueous infusion of tissue of the small intestine of the pig had but slight hydrolytic action on starch, whereas contact with the tissue itself produced pronounced hydrolysis.

Vines[7] (1891) found that the turbid extract of green leaves when merely strained was much more active than the clear extract obtained by filtration.

Kastle and Loevenhart,[8] in their work on lipase, macerated the pancreas with sand, then mixed the mass with water, and simply strained through linen or cotton cloth. They report:

> The effect of filtration was invariably to diminish the activity of the solution. This was proved experimentally for aqueous and glycerin extracts of both pancreas and liver. The same was also found to hold for the pancreatin of Parke, Davis & Co. A two per cent. solution of this preparation is yellowish in color and is turbid. In this shape it possesses weak but measurable lipolytic power. On filtering a great many times, a clear yellowish solution is obtained, which possesses only a trace of its original lipolytic activity.

To show further the effect of filtration on a liver extract, they cite the following:

Before filtering, 1 c.c. of a 10 per cent. liver extract was found to hydrolyze 6.28 per cent. of ethyl butyrate in fifteen minutes at 40°. After filtering repeatedly through the same filter paper, it hydrolyzed only 2.76 per cent., showing a falling off in activity of over one-half as the result of filtration. The removal of lipase along with suspended matter in the solution was observed in still another connection. When an active pancreatic extract acts on ethyl butyrate at 40°, the butyric acid produced soon coagulates the proteids in the extract. On filtering it was found that the filtrate possessed little or no lipolytic power, whereas the proteid coagulum was found to be exceedingly active.

PAPER FILTRATION OF PTYALIN.

In order to determine whether or not ptyalin could be removed from saliva by filtration through paper, the following experiment was carried out:

Two hundred cubic centimeters of saliva were collected by mastication of paraffin, and subjected to filtration through eight double filters of Schleicher and Schüll's hardened filter paper, No. 575. These filters were arranged as follows: In each of two funnels were placed two folded filter papers. The funnels were arranged one above the other on a ring stand, so that the stem of the upper funnel touched the side of the filter paper in the lower one. The stem of the lower one passed into an Erlenmeyer flask. Thus the saliva poured into the upper funnel passed through four thicknesses of paper before reaching the flask. This filtrate, designated as "2×2," then passed through a second set of filters similarly arranged and gave filtrate "4×2," the passage of which through another set of double filters produced filtrate "6×2," which in turn filtered as before, through a fourth set of new double papers, gave as final filtrate, "8×2." In all of our paper-filtration experiments, the enzyme solution was filtered through eight double filters of hardened paper.

Previous to the filtration through the eight double filters, the saliva was filtered through one thickness of ordinary filter paper (S. & S., No. 595) to remove the mucin and solid particles which otherwise retarded the subsequent filtration.

In order to ascertain the decrease in diastatic activity, a definite quantity of each filtrate was added to a definite volume of a starch solution, and the resulting mixture was kept at a fixed temperature in a water bath. From time to time a portion of the liquid was removed and tested with iodine until the achromic point was reached.

A preliminary trial indicated the necessity of using but small portions of the successive filtrates in order to determine the pro-

gressive decrease in activity of the filtered solution, since in concentrated solutions the action of ptyalin is evidently not directly proportional to the amount of the enzyme present. The following experiment will make this clear:

Two hundred cubic centimeters of saliva, previously passed through one thickness of ordinary filter paper, were subjected, as above described, to eight successive double filtrations; each filtrate and a portion of unfiltered saliva which was set aside as control, were tested by adding 0.5 c.c. of each to 30 c.c. of a 1 per cent. solution of starch, and the mixture was allowed to digest at 34–37°. A similar set was made, differing only in that the control and each filtrate were tested by adding 0.1 c.c. of each to 30 c.c. of a 1 per cent. starch solution. The filtration of the first set consumed about two hours, while that of the second required three and one-quarter hours. The following table gives the results, indicating the number of minutes required in each test to reach the achromic point:

TABLE I.

	Digested with 0.5 c.c.	Digested with 0.1 c.c.
Control	10 min.	30 min.
"2 × 2" filtrate	10 min.	42 min.
"4 × 2" filtrate	10 min.	50 min.
"6 × 2" filtrate	8 min.	62 min.
"8 × 2" filtrate	6 min.	Pink in 82 min.

The above, then, indicates that, although ptyalin is removed from its solution by filtration, this fact is not made manifest unless the quantity of saliva employed in testing is small enough to prevent the masking of the loss by the activity of the remaining enzyme. For this reason in the following experiments diluted saliva was employed.

EXPERIMENT I.

Twenty cubic centimeters of saliva were collected by mastication of paraffin and filtered through one thickness of ordinary filter paper. This filtrate was diluted to 200 c.c. with distilled water. Filtration was then carried out as above described. A portion of the original diluted saliva was set aside (10 per cent. solution) in a test tube at the same temperature and light conditions as obtained for the filtering saliva. One cubic centimeter of this control solution was added to 30 c.c. of the starch solution at the same time that 1 c.c. of each successive filtrate was similarly treated. A filtered 1 per cent. starch solution containing glycerin was employed for these tests. The filtering liquid and the control were protected from light by black cloth. The progress of digestion in each case was noted by removing from each tube from time to time 2 c.c. of the solution and testing this with a dilute

iodine solution. The pipette used was rinsed thoroughly each time with boiling distilled water.

The time required to reach the achromic point is indicated in the following table:

TABLE II.

1 C.C. 10 PER CENT. SALIVA + 30 C.C. OF STARCH SOLUTION. DIGESTED AT 34°.

Control	Achromic Point	Filtrate from	Achromic Point
"2 × 2"	20 min.	"2 × 2"	20 min.
"4 × 2"	20 min.	"4 × 2"	20 min.
"6 × 2"	20 min.	"6 × 2"	25 min.
"8 × 2"	20 min.	"8 × 2"	Deep purple in 1 hr. 45 min.

The time consumed in filtration was two hours and ten minutes.

TABLE III.

0.5 C.C. SALIVA + 30 C.C. OF STARCH SOLUTION, DIGESTED AT 34°.

(This experiment is like the preceding save that 0.5 c.c. of control and of each filtrate was added to 30 c.c. of the starch solution.)

Control	Achromic Point	Filtrate from	Achromic Point
"2 × 2"	40 min.	"2 × 2"	48 min.
"4 × 2"	40 min.	"4 × 2"	54 min.
"6 × 2"	36 min.	"6 × 2"	70 min.
"8 × 2"	36 min.	"8 × 2"	Blue in 1 hr. 30 min. Purple in 18 hrs.

The duration of the filtration process in this case was two hours and thirty minutes.

Further experiments gave the same results as the preceding, showing without a doubt that saliva is relieved of its ptyalin by filtration through hardened filter paper.

To what, then, is this result due? Three answers present themselves: the molecules of ptyalin, as in the case of a precipitate, may be mechanically retained by the filter; or the aeration or the surface action, if any, may have a destructive effect; or, finally, the ptyalin may have entered into firm combination with the material of the filter paper, *i. e.*, it may have been *fixed*.

EXPERIMENT III. (BERKEFELD FILTRATION.)

To determine whether the paper acted by mechanical retention, the following experiment was carried out:

A.—20 c.c. of saliva, filtered through a single ordinary filter paper, were diluted to 200 c.c. with distilled water. The entire quantity, less 5 c.c. which were set aside as control, was filtered through a No. 5 Berkefeld filter (1 × 8

inches) at room temperature. The diastatic action of the filtrate was tested and compared with that of the control solution.

B.—Same as A, save that filter was warmed to 50° by repeated washings with distilled water warmed to 50° and 195 c.c. of 10 per cent. saliva warmed to 50° filtered through bougie. Control of unfiltered saliva warmed to 50°. Tested filtrates and controls as before. The time required to produce achromicity is indicated in the table below:

TABLE IV.

0.5 C.C OF SALIVA + 30 C.C. STARCH SOLUTION, AT 34°.

	Temperature	Filtration Pressure	Rate of Filtration	Achr. Pt. of Filtrate	Achr. Pt. of Control
A.....	Room	740 mm.	7.5 min. per 100 c.c. *ca.*	40 min.	40 min.
B.....	50°	720 mm.	7 min. per 100 c.c. *ca.*	40 min.	40 min.

This disposes of the idea of the mechanical retention of the molecules. It is quite inconceivable that the molecules should be retained by the comparatively large pores of the filter paper, and be allowed unimpeded passage through the pores of the Berkefeld filter which denies passage to minute bacteria. Some factor other than mechanical retention has been at work.

EXPERIMENT IV.

To determine the effect of aeration the following experiment was performed:

Over the narrowed end of a piece of amber glass tubing 46 cm. long and 1½ cm. wide was slipped a piece of rubber tubing 5 cm. long. This tube was provided with a capillary glass tip and with a Hofmann clamp. The amber glass tube was then filled to a depth of about 35 cm. with clean glass beads, which were prevented from escaping from the tube by a thin plug of glass wool. The tube was then clamped in an upright position to a ring stand. The Hofmann clamp was closed, and about 25 c.c. of 10 per cent. saliva (filtered through ordinary filter paper before dilution) were poured into the tube. A rubber stopper carrying an L-shaped glass tube closed the upper end of the tube, and this L was connected by rubber tubing with an aspirating pump. Suction was started and the Hofmann clamp was opened sufficiently to allow a vigorous, but not too violent, aeration of the contents of the tube. This aeration was allowed to continue for twenty-four hours, the amylolytic action of the liquid being tested before aeration was begun, also at the end of three, six, nine, and twenty-four hours respectively. A control of unaerated 10 per cent. saliva set aside at room temperature under a black cloth was likewise

tested at the same intervals. Saliva, control and aerated, was tested by adding 0.5 c.c. to 30 c.c. of 1 per cent. starch solution, and digestion was allowed to proceed at 35°. The time required to produce achromicity is indicated in the following table:

TABLE V.

Control	Achromic Point	Saliva Aerated for	Achromic Point
Original...	32 min.	.....	.....
3 hrs.....	20 min.	3 hrs.....	24 min.
6 hrs.....	22 min.	6 hrs.....	24 min.
9 hrs.....	38 min.	9 hrs.....	38 min.
24 hrs.....	24 min.	24 hrs.....	24 min.

The slowness of digestion in the nine-hour set was probably due to the fact that the digestion temperature was 32°, whereas that of the other sets was 35°.

It is evident from the above that aeration, even for twenty-four hours, is without appreciable injurious effect on a 10 per cent. saliva.

Szumowski[9] has demonstrated the fixation of enzymes (ptyalin, rennin, pepsin, trypsin) by fibrin, and Effront[10] states that enzymes are fixed by different substances, such as silk and fibrin. Chevreul[11] (1853) tested the fixing or mordant action of wool, cotton, and silk on such substances as sodium chloride, sulphuric acid, mercuric chloride, hydrochloric acid, lime water, baryta water, alum, barium nitrate, lead nitrate, and ferrocyanide of potassium. To quote from one experiment:

> Thus cotton absorbs $HgCl_2$ and water in the proportion of the solution, and moreover, it retains a portion of it with so much force that, after having been washed until the wash-water no longer precipitates $AgNO_3$, it is colored by a solution of H_2S and it acts otherwise than pure cotton on the coloring principles of cochineal, logwood, and madder.

"The action of solids in altering solutions," says Chevreul, "shows how filters can act in a chemical manner on the liquids which traverse them."

Musculus (*loc. cit.*) shows that if urine in active alkaline fermentation be filtered through paper, this paper, after being washed until the wash-water is free from any alkaline reaction and dried at 35 or 40°, retains, even for months, a something capable of exciting alkaline fermentation of urea. This something he considered to be "torulæ," retained in the pores of the

paper. It is possible, in view of the demonstration by Miquel (*loc. cit.*) of the solubility of urase, that the filtered paper had fixed the urase.

O'Shea[33] found that lead was retained by various filter papers (English, French, and German make) on filtering lead salts. An experiment shows that a S. & S. filter, 589, absorbed from 50 c.c. of lead acetate 0.12 mg. of lead, 0.02 mg. of which was removed by a first and none by a second washing. On refiltering 0.24 mg. was absorbed, none of which was removed by subsequent washing.

That hardened filter paper fixes ptyalin is proved by the following experiments:

EXPERIMENT V.

In the aeration tube above described were placed about 1,500 sq. cm. of hardened filter paper, cut into bits about ½ cm. sq. and then crumpled. The papers in the tubes were first washed with distilled water and then with 10 per cent. saliva (filtered through ordinary filter paper before dilution), after which 35-40 c.c. of the 10 per cent. solution were added and aerated over the paper bits, the same as before over beads, and the diastatic activity was tested at intervals of three, six, nine, and twenty-four hours. A portion of unaerated saliva was set aside at room temperature under a black cloth for control and was tested at the same intervals.

The time required to produce achromicity is shown in the following table:

TABLE VI.

0.5 C.C. 10 PER CENT. SALIVA + 30 C.C. 1 PER CENT. STARCH SOLUTION AT 35°.

Control	Achromic Point	Saliva Aerated for	Achromic Point
Original	36 min.	.....	.....
3 hrs.	36 min.	3 hrs.	64 min.
6 hrs.	32 min.	6 hrs.	60 min.
9 hrs.	32 min.	9 hrs.	56 min.
24 hrs.	32 min.	24 hrs.	60 min.

Although aeration over beads produced no change in activity of saliva, aeration over paper did. The saliva was now drained off, and the papers were washed by rinsing and aerating with distilled water repeatedly, until the wash-water no longer exhibited a trace of ptyalin.

A number of shreds of paper were then removed from the tube, rinsed in a beaker of water, and added to 30 c.c. of a 0.1 per cent. starch solution. To another tube containing 30 c.c. of the starch solution was added an equal quantity of fresh crumpled bits of hardened filter paper, washed in distilled water. This latter served as control. Both were placed in a thermostat at 35°. Portions of contents of each tube responded to iodine with blue coloration at the end of an hour. Absence from laboratory prevented further test until at the end of forty hours, at which time the *starch in the tube*

which contained paper aerated with saliva gave achromic reaction with iodine, while starch in control tube showed no inversion whatever.

At this time the water was drawn off from the washed papers which remained in the tube, and glycerin was added. Aeration was then proceeded with for thirty minutes, at the end of which time the glycerin was withdrawn and 10 c.c. added to 10 c.c. of the 0.1 per cent. starch solution. This was placed in water bath at 35°, and portions were tested with iodine at intervals indicated below, with the following results:

At the end of fifteen minutes the color produced by the iodine was blue. When next tested at the end of twenty-five minutes, no color reaction was obtained. This shows that, as will be borne out in subsequent experiments, the glycerin had extracted the ptyalin which had been retained by the filter paper.

EXPERIMENT VI.

The saliva was aerated over paper as in the preceding experiment. The aerated saliva and control were tested at the end of three hours and fifteen and one-half hours.

TABLE VII.

0.5 C.C. OF 10 PER CENT. SALIVA + 30 C.C. OF 1 PER CENT. STARCH SOLUTION AT 35.5°.

Control	Achromic Point	Saliva Aerated for	Achromic Point
3 hrs.....	20 min.	3 hrs.....	20 min.
15½ hrs.....	20 min.	15½ hrs.....	20 min.

In the preliminary filtration experiments it was seen that, although the liquid experimented with loses some of its ptyalin, the activity of the remaining ptyalin hides this fact. That this is also the case with saliva aerated in Experiment VI is shown by the following:

The paper bits in the tube were washed with distilled water until all traces of free ptyalin were removed, and the paper was then taken from the tube. A number of the bits were added to 30 c.c. of a 0.1 per cent. starch solution and placed in the thermostat at 36°. A corresponding number of fresh bits were likewise added to 30 c.c. of starch solution for control. The experiment was further controlled by placing in the thermostat a third tube containing 15 c.c. of the last wash-water, added to 15 c.c. of the starch solution. Portions of the liquid in each tube were tested from time to time with iodine, with the following results:

Control wash-water + starch solution — blue in 2½ hrs.
Control paper + starch solution — blue in 2½ hrs.
Paper aerated with saliva + starch solution — achromic in 2½ hrs.

At the end of nineteen hours both controls gave purple reaction with iodine.

EXPERIMENT VII.

The bits of paper from Experiment VI not employed in making the above test were kept in refrigerator for twenty-one hours. They were then returned to the washed tube, which was labeled *A*. In another similar tube, labeled

B, was placed an approximately equal amount (about 1,000 sq. cm.) of paper in crumpled bits. To each tube was added 70 c.c. of 10 per cent. saliva, filtered before dilution through ordinary filter paper. The contents of the tubes were then aerated side by side at equal rates of aeration for twenty-four hours, and both were controlled by an unaerated 10 per cent. saliva kept at room temperature under a black cloth. The control was tested at the same intervals as the aerating saliva. The saliva was extremely active, and for that reason in the following tests only 0.1 c.c. of saliva was employed.

TABLE VIII.

0.1 C.C. SALIVA + 30 C.C. STARCH SOLUTION AT 36°.

Tube *A* contained old saliva papers.
The *B* contained fresh saliva papers.

	Control Saliva	Saliva from *A*	Saliva from *B*
3 hrs.....	Achromic in 60 min.	Achromic in 64 min.	Achromic in 66 min.
6 hrs.....	Achromic in 60 min.	Achromic in 74 min.	Achromic in 74 min.
24 hrs.....	Pink in 80 min.	Achromic in 66 min.	Achromic in 66 min.

The starch used in testing the twenty-four-hour control was twenty-four hours older than that used in testing the twenty-four-hour sets of *A* and *B*, which may account for the irregularity apparent in twenty-four-hour set.

This experiment was performed for the purpose of a comparison of papers presumably ptyalin-saturated (*A*), with fresh papers (*B*) as regards their action on 10 per cent. saliva. Both appear to have similar effect, a slightly greater avidity for ptyalin being suggested by the fresh papers at the end of three hours, after which they too appear to be saturated. A better comparison is afforded in Table XIII.

The paper bits in both tubes were now thoroughly washed until no trace of free ptyalin remained. The action of the papers was then tested as before.

TABLE IX.

Control	Iodine Reaction	Papers from	Iodine Reaction
Fresh paper control	Blue in 1 hr.		
Wash-water control (*A*).....	Blue in 1 hr. Red in 2 hrs. Achromic in 19 hrs.	*A* (old saliva papers)	Achromic in 1 hr.
Wash-water control (*B*).....	Blue in 2½ hrs.	*B* (fresh saliva papers)	Achromic in 1 hr.

In the meantime the papers in *B* were extracted by aeration with glycerin for thirty minutes. The test of the aerated glycerin for diastatic activity as compared with fresh glycerin resulted as follows:

15 c.c. of paper aerated glycerin + 15 c.c. 0.1 per cent. starch solution produced achromicity in 18 hours.

15 c.c. of fresh glycerin + 15 c.c. of 0.1 per cent. starch solution produced no inversion whatever in 18 hours.

EXPERIMENT VIII.

Added fresh glycerin to papers in *B* and fresh distilled water to *A*, and aerated for twenty-four hours. Tested wash-water of *A* and glycerin of *B* as before, controlling with fresh water and fresh glycerin.

TABLE X.

15 C.C. OF WATER AND OF GLYCERIN + 15 C.C. 0.1 PER CENT. STARCH SOLUTION AT 36°.

Control	Color Produced by Iodine	Wash Liquid	Color Produced by Iodine
Water + starch	Deep purple in 4½ hrs.	Water (*A*).....	Deep purple in 4½ hrs.
Glycerin + starch..	Blue in 1 hr.	Glycerin (*B*).....	Achromic in 1 hr.

Portions were tested every fifteen minutes in the above tests. Thus with twenty-four hours' washing, water removed none of the retained ptyalin whereas glycerin extracted it.

The tubes were then drained and rinsed with distilled water, after which fresh water was added to *A* and fresh glycerin to *B*. They were aerated then for forty-eight hours, and tested as before.

TABLE XI.

Control	Color Produced by Iodine	Wash-Liquid	Color Produced by Iodine
Water + starch....	Blue in 4½ hours	Water (*A*).....	Blue in 4½ hours
Glycerin + starch..	Blue in 4½ hours	Glycerin (*B*).....	Achromic in 4½ h.

Even this attempt at extraction (seventy-two hours' aeration) with water failed to remove the ptyalin from its close retention by the filter paper, although the paper yielded its ptyalin readily to the glycerin. That the paper still retained its ptyalin is further shown by the following:

The glycerin was thoroughly removed from *B* by repeated washings with distilled water. *A* was drained, and a third control tube was set up, containing fresh papers, approximately equal in quantity to those in *A* and *B*. Water was placed in tubes *A*, *B*, and *C*, and all were aerated for one and three-quarter

hours. A portion of the papers was then removed from *A* and *B*, and the amylolytic action tested: tests were controlled by paper bits from *C*. Controlled further with wash-water from *A* and *B* by adding 15 c.c. of each to 15 c.c. of 0.3 per cent. starch solution and digesting at 36°.

TABLE XII.

Control	Color Produced by Iodine	Saliva Paper	Color Produced by Iodine
Paper control.....	Blue in 3½ hrs. Purple in 18 hrs.		
			Blue in 3½ hrs.
Wash-water of *A*..	Blue in 3½ hrs. Purple in 18 hrs.	*A* (water-washed)	Purple in 6 hrs. Achr. in 18 hrs.
Wash-water of *B*..	Blue in 3½ hrs. Purple in 18 hrs.	*B* (glyc. extracted)	Purple in 3½ hrs. Achr. in 18 hrs.

The liquids were examined only at the times indicated in the table.

After enough liquid was removed from each tube for the preceding test, the tubes and contents were rinsed with 2 per cent. saliva (the latter being filtered, before dilution, through a single thickness of ordinary filter paper). To each tube there were then added 25 c c. of the 2 per cent. saliva, and the contents were aerated for twenty-four hours, with interruptions at the end of three and six hours as before. The tubes contained approximately equal quantities of paper and were aerated side by side at equal rates.

Tube *A* contained old saliva papers, water-washed.

Tube *B* contained old saliva papers, glycerin-extracted.

Tube *C* contained fresh papers, water-washed.

At the end of three, six, and twenty-four hours, 1 c.c. aerated 2 per cent. saliva from each tube was added to 30 c.c. starch solution and digested at 34° to 37°. The results are tabulated below:

TABLE XIII.

Aeration	Color Produced by Iodine			
	Control	Tube *A*	Tube *B*	Tube *C*
3 hours.....	Achr. in 50 min.	Achr. in 7 hrs.	Achr. in 6 hrs.	Blue in 7 hrs.
6 hours.....	Achr. in 50 min.	Pink in 8 hrs.	Achr. in 6½ hrs.	Blue in 1¼ hrs.
24 hours.....	Achr. in 50 min.	Blue in 3 hrs.	Purple in 3 hrs.	Blue in 3 hrs.

It is seen from the above that with sufficient dilution of saliva all of the ptyalin can be retained by the filter paper in the aeration tubes, just as it can be removed from like solutions by filtration. The paper bits in *A* and *B* already contained fixed ptyalin,

and on that account could not remove all the ptyalin from the 2 per cent. saliva, although a glance at the table shows that a very considerable quantity was removed. The paper in *C* so effectually removed the ptyalin that at the end of three hours' aeration 1 c.c. of the liquid could produce no effect on 30 c.c. of 1 per cent. starch solution in seven hours, whereas a corresponding quantity of the control saliva completely inverted 30 c.c. of the starch solution in fifty minutes.

That the ptyalin was not destroyed is made evident by the following:

The paper bits in Tubes *A*, *B*, and *C* were thoroughly washed as before, and approximately equal quantities of each were immersed in test-tubes containing 30 c.c. of 1 per cent. starch solution. Control tests were made with fresh paper as before. Wash-water controls as before. The tests with the wash-waters were checked with distilled water. The digestion was carried on at 35°.

TABLE XIV.

Water Control	Color Produced by Iodine	Paper Bits	Color Produced by Iodine
Distilled water.....	Blue in 18 hrs.	Control.....	Purple in 18 hrs.
Wash-water *A*.....	Blue in 18 hrs.	Tube *A*.....	Pink in 3½ hrs. Achromic in 18 hrs.
Wash-water *B*.....	Blue in 3½ hrs. Pink in 18 hrs.	Tube *B*.....	Achromic in 3½ hrs. Blue in 1 hr.
Wash-water *C*.....	Blue in 18 hrs.	Tube *C*.....	Pink in 3½ hrs. Achromic in 18 hrs.

The liquids were examined only at times indicated in the table.

EXPERIMENT IX.

Two sheets of S. & S. hardened filter paper, No. 575, diameter 24 cm., were cut up into small bits (about 1 cm. square), crumpled, and placed in an aeration tube, which we will designate as *A*. A similar quantity of papers was placed in a second tube *B*. In a third tube *C* were placed glass beads, the height of the column of which equaled that of the paper in *A* and in *B*. The saliva was filtered through a single thickness of ordinary filter paper and 4 c.c. of it was diluted to 200 c.c. with distilled water. After rinsing the tubes with this 2 per cent. saliva, 60 c.c. of the same were placed in *A* and *B* respectively, while *C* received 40 c.c.

Tubes *A* and C were connected with the pump and aerated at the same rate. Tube *B* remained unaerated for two hours, and was then aerated for five minutes, after which it remained unaerated for two hours, and then was again aerated for five minutes. It now remained unaerated for the next eighteen hours, after which it was aerated for fifteen minutes. The saliva in

A, *B*, and *C* was tested at three-hour intervals, and the tests were controlled with unaerated 2 per cent. saliva, which was kept at room temperature under a black cloth.

TABLE XV.

1 C.C. OF 2 PER CENT. SALIVA + 30 C.C. 1 PER CENT. STARCH SOLUTION AT 38°.

(For the six-hour test a new starch solution was prepared.)

SALIVA	COLOR PRODUCED BY IODINE		
	3 Hours	6 Hours	24 Hours
Control.....	Achr. in 26 min.	Achromic in 50 min.	Achromic in 38 min.
A.....	Achr. in 62 min.	Deep purple in 1 hr.; achr. in 16½ hrs.	Purple in 3 hrs.; achr. in 4 hrs.
B.....	Achr. in 62 min.	Blue in 1 hr.; achr. in 16½ hrs.	Blue in 4 hrs.; deep purple in 24 hrs.
C.....	Achr. in 26 min.	Achr. in 50 min.	Achr. in 32 min.

From the above table it will be seen that aeration over beads has no effect, and that fixation is more rapid when the liquid is not continuously agitated.

The papers in *A* and *B* were then washed by aerating for twenty-four hours with distilled water, which was frequently changed. The inverting action of the washed papers was tested as before.

TABLE XVI.

Paper Bits	Color Produced by Iodine
Control.....	Blue in 5¼ hrs.
Saliva paper *A*...	Blue in 2 hrs.; light pink in 5¼ hrs.
Saliva paper *B*...	Blue in 2 hrs.; achromic in 5¼ hrs.

DIALYSIS OF PTYALIN THROUGH COLLODION SAC.

Gorsline,[12] working in this laboratory, showed that such substances as starch and albumin can pass through the collodion membrane, and suggested the possibility of dialyzing enzymes. According to Rodet and Guéchoff,[13] the collodion wall does not act as a perfect filter. They say:

To be acquainted with the value of collodion sacs in bacteriological experiments it is quite necessary to know whether the membrane of collodion constitutes a filtering septum, retaining only formed particles, and allowing to pass indiscriminately all soluble matters, or whether, endowed with properties intermediate between those of such a filtering wall and those of a semi-permeable membrane, it allows soluble substances to pass more or less, and in a varying degree, according to their nature and the size of their molecules.

We have wished first to see by an experiment *in vitro* if collodion is perfectly permeable to bodies of different molecular weights, such as sugar, peptone, albumin. . . . . Three similar sacs were filled with distilled water, and were plunged into test-tubes containing several cubic centimeters, one of a solution of glucose, another of a two per cent. aqueous peptone solution, and the third of blood serum. After twenty-four hours' sojourn at laboratory temperature special reactions were noticed in the contents of the sacs. The contents of the first reduced Fehling's solution abundantly; the sugar had traversed the wall of the collodion. The third sac gave the albumin reactions; but the content of albumin was very feeble, estimated practically 500 times less than that of the serum in which the sac was immersed; the albumin had traversed the wall but feebly. As to the contents of the second sac, the peptone reaction was not found there; it is, however, quite reasonable to suppose that it had passed, but in a quantity insufficient to be detected by the reagents. This experiment shows that collodion does not comport itself as a perfect filter.

It is quite reasonable to suppose that the sacs used by Messrs. Rodet and Guéchoff in this experiment were not of the same degree of thickness—that the sac used in the peptone dialysis was thicker than that used in the dialysis of albumin. If albumin readily traverses the collodion wall, it is quite evident that the relatively simpler peptone should do likewise. The inequality of the sacs is probably due to the fact that those used were of a commercial variety, as indicated below. To continue:

Further, we have made two experiments on animals, one with diphtheria toxin, the other with an alkaloid which it may be assumed must readily traverse the membrane—strychnine.

Experiment with diphtheria toxin: A guinea pig (600 g.) received in its peritoneal cavity a collodion sac (commercial) inclosing 2 c.c. of broth containing 5 g. of diphtheria toxin, fatal at 0.05 g. for a 250 g. guinea pig. It survived. Another (500 g.) received an intraperitoneal injection of 0.25 g. (a dose one-half less than that of the preceding) of the same toxin. It died in less than forty hours.

A dose of diphtheria toxin, much greater than the fatal dose, is thus perfectly tolerated in a collodion sac.

Experiment with strychnine: A guinea pig (540 g.) received July 7 in the peritoneal cavity a collodion sac (commercial) with 0.75 c.c. of an aqueous solution containing ¾ mg. of sulphate of strychnine. The following day it showed nothing in particular; no contractions. It died on the 14th, but its death was not due to strychnine. The operation wound had been opened, and allowed the intestine to protrude; the body was supple; on the evening of the 13th there had been no contraction. The contents of the sac, withdrawn and injected subcutaneously into another guinea pig, did not cause death.

Consequently the toxin diffused out of the sac, but so gradually as to be tolerated.

. . . . . We are convinced that the collodion wall is far from being as permeable to dissolved substances as bacteriologists have admitted up to the present, and as a consequence the different interpretations of experiments made on this principle deserve to be held in doubt.

These experiments are of interest in that they call attention to the imperfect permeability of the collodion wall—a fact which our own experiments will further substantiate. We are inclined to believe, however, that with sufficiently thin sacs the dialysis in the above experiments would have been more marked.

We wish further to call attention to the experiments of Crendiroupoulis and Ruffer,[14] who find the toxin of pyocyaneus is partially dialyzable through the collodion wall, but not through walls of too great thickness. They state further that the comparison of the toxin filtered through the bougie with the toxin dialyzed through collodion shows a difference in pathogenic energy quite in favor of the former. They have, however, been able to produce with dialyzed toxin all the symptoms of pyocyaneus intoxication without exception, provided the quantity injected be sufficiently large.

The sacs employed in the following experiments were made according to the method described by Gorsline (*loc. cit.*). They were about $2\frac{1}{2}$ inches in length and $\frac{1}{2}$ inch in diameter, and were made by turning the tube used in making the sacs twice in dilute collodion, producing a sac of extreme thinness. These sacs were free from air bubbles, opaque areas, or flaws of any nature, were practically invisible when immersed in water, and collapsed immediately on emptying. Each sac was tested for possible leaks by immersing while empty in distilled water and distending with air under pressure. Those sacs showing evidences of leakage were discarded.

EXPERIMENT X.

Seven thin collodion sacs were prepared as above described. Each was mounted on a glass tube, some three inches in length, made by cutting off the end of a small test-tube. The sac was slipped over the end of this tube for a distance of a half-inch, and then contracted on the glass by rotation over a narrow flame, as described by Novy.[15] This joint was finally recoated

with collodion. As stated above, each sac was tested for leaks before it was used.

A quantity of saliva was filtered through a single thickness of ordinary filter paper. In each of six sacs, labeled 1–6 inclusive, were placed 5 c.c. of this saliva. In the control sac, marked *C*, were placed 5 c.c. of same saliva, previously boiled for five minutes to destroy the enzyme. Each sac was immersed in 20 c.c. (Sac 5 in 25 c.c.) of a 0.01 per cent. starch solution, contained in a test-tube on foot. In no sac did the liquid reach the joint, thereby insuring impossibility of capillary escape of liquid from sac.

The tubes containing these sacs were set aside in an incubator at 37°. At the end of three hours 2 c.c. of the liquid were withdrawn from each tube and tested with several drops of dilute iodine with the following results:

TABLE XVII.

Contents of Tube	Reaction Produced by Iodine
C	Blue
1	Achromic
2	Achromic
3	Achromic
4	Achromic
5	Pink
6	Achromic

The contents of Tube 5 and of the control tube were again tested at the end of the fourth hour; the latter gave a blue, starch reaction, while the former was achromic.

The sacs were again tested and found to be free from leaks. The only way by which the inversion of the starch could have been brought about was by the dialysis of the ptyalin through the collodion membrane.

Further similar experiments gave identically the same results and clearly demonstrated that ptyalin will dialyze through the collodion membrane.

The next point was to determine whether or not ptyalin could be filtered through such a membrane. For this purpose the following experiment was made:

EXPERIMENT XI.

A thin collodion sac was attached, in the manner above described, to a glass tube passing through a rubber stopper. The joint was further strengthened by winding with a silk thread, and again recoating with collodion. The sac was of the same thickness as those used in the above dialysis experiments. The empty sac was tested under water by forcing air into it at 3 inches' pressure, and was found to be free from leaks. The sac thus mounted was placed in a test-tube on foot, provided with a side arm. The rubber stopper and the mouth of the tube formed an air-tight joint. Five cubic centimeters of the undiluted saliva, previously filtered through a single thickness of ordinary filter paper, were then introduced into the sac. The side arm of tube was connected with a gently working aspirator, and a suction pressure of 2½ inches was maintained for one and one-half hours. During this time droplets of perspiration appeared on the outer side of the distended sac, slowly

became confluent, collected in drops at the lower end of the sac, and fell to the bottom of the test-tube. In this way 1.5 c.c. of the contents of the sac were drawn through the wall and collected at the bottom of the test-tube. The filtering liquid and the control were protected during the process against the action of light by covering with black cloth.

One cubic centimeter of this filtrate was added to 30 c.c. of a 1 per cent. starch solution. An equal quantity of the control or paper-filtered saliva was added to a similar amount of starch solution. A third tube, containing 30 c.c. of the starch solution, served as a blank control. These tubes were set aside at 37–38°, and portions of the liquid were removed, from time to time, from each tube and tested with iodine. The result was as follows:

1 c.c. control + 30 c.c. starch solution — achromic in 20 min.
1 c.c. filtrate + " " " — blue in 22 hrs.
Starch control — blue in 22 hrs.

This experiment shows that the ptyalin was completely retained by the collodion filter.

The filter sac employed above was well washed, and then 5 c.c. of fresh filtered saliva were introduced. It was then immersed in 20 c.c. of 0.01 per cent. starch solution and placed in an incubator at 37°. The experiment was controlled with a sac similarly immersed containing 5 c.c. of boiled saliva. In a control test with iodine at the beginning of the dialysis the starch gave a deep blue color. At the end of three hours the starch in both tubes showed complete inversion. The fact that the control or heated saliva behaved thus showed that the boiling was insufficient to destroy its activity.

The sacs were again tested and found free from leaks. Thus, the same sac which refused passage to ptyalin, when used as a filter, allowed the enzyme to pass through it readily by dialysis. This fact is further substantiated by the following similar experiment:

Some saliva was placed in a new thin collodion sac and aspirated as before. Three and two fifths c.c. of a water-clear liquid filtered through in one and one-half hours at a pressure varying from 2¾ to 3¼ inches. This filtrate was tested as before at 38°.

1 c.c. of control saliva + 30 c.c. of 1 per cent. starch solution—achromic in 30 min.
1 c.c. saliva filtrate + 30 c.c. 1 per cent. starch solution—blue in 45 hrs.
Blank control (30 c.c. of starch solution) blue in 45 hrs.

The control was made with the unfiltered saliva remaining in the sac. Therefore the activity of the ptyalin was not destroyed by the action of the collodion wall or by the pressure. The sac was tested before and after use at a pressure of 3¼ inches, and was found to be free from leaks. It was then employed for a dialysis test, for which purpose 5 c.c. of fresh saliva were introduced into the sac, which was then immersed in a test-tube on foot containing 20 c.c. of a 0.01 per cent. starch solution. A control sac similarly immersed, containing 5 c.c of saliva previously boiled for five minutes, was also set aside. The tubes were placed in the incubator at 37°. An initial control test with iodine gave a deep-blue color. At the end of one hour and at the end of one hour and forty minutes portions of starch were removed from each tube and tested with iodine with the following results:

TABLE XVIII.

	Control	1 Hour	1 Hr. 40 Min.
Used sac	Blue	Pink	Achromic
Control sac	Blue	Blue	Blue

The foregoing experiments demonstrate the ready dialysis of ptyalin through the collodion sac, and the perfect impermeability of the latter to ptyalin when used as a filter. This impermeability is not due to fixation, for if the collodion wall could fix ptyalin, dialysis could not occur.

The fact that ptyalin is retained from saliva on passage of the latter through hardened filter paper; that it is not retained by the Berkefeld bougie: that it is not affected by aeration: that paper bits which have been immersed in saliva, when washed entirely free from saliva, will yet invert starch; that this paper will not give up this property on washing with water, nor yield to the water any diastatic property, and that it yields its retained ptyalin readily to glycerin, goes to show that ptyalin is *fixed* by hardened filter paper.

TAKA DIASTASE.

FILTRATION THROUGH PAPER.

The solutions of taka diastase (Parke, Davis & Co.), of a dilution of 1:4,000 were first filtered through a single thickness of ordinary filter paper. They were then subjected to filtration through eight double filters of hardened filter paper, and each filtrate was tested in the manner as given under ptyalin. In the several filtrations tried the "8 × 2" filtrate showed itself but little, if indeed at all, weaker in activity than the original control. Inasmuch as this 1:4,000 solution was but half as strong as a 10 per cent. solution of ordinary saliva, it was not deemed necessary to work with greater dilutions.

Experiments in the aeration tube likewise failed to demonstrate any fixation, although the activity of the enzyme was decreased by the aeration. This weakening was due either to the aeration itself, or to the surface action of the paper and the beads over which it was aerated, or to both.

The enzyme passed through the Berkefeld filter at vacuum pressure as readily as it passed through the "8 × 2" filters.

DIALYSIS.

The following, one of several similar experiments, indicates the dialysability of taka-diastase:

Four thin collodion sacs were prepared and mounted as previously described, and labeled *C*, 1, 2, and 3. In each of 1, 2, and 3 were placed 5 c.c.

of a 1:1,000 distilled water solution of taka diastase, previously filtered through a single thickness of ordinary filter paper. In *C* were placed 5 c.c. of the same solution, previously boiled for five minutes. This served as control solution. Each sac was immersed in a test-tube on foot containing 20 c.c. of a 0.01 per cent. starch solution, and the whole was then placed in the incubator at 36° for the purpose of dialysis. A control test of the starch before immersion of the sacs gave a deep-blue coloration on addition of iodine. Portions of 2 c.c. were removed from each of the test-tubes at the end of three, six, nine, and twenty-four hours, and were tested with dilute iodine to observe progress of digestion, if any. The results obtained are given in the following table:

TABLE XIX.

Time	Tube *C*	Tube 1	Tube 2	Tube 3
3 hrs.	Blue	Blue	Blue	Blue
6 hrs.	Blue	Light blue	Light blue	Light blue
9 hrs.	Blue	Pink	Pink	Pink
24 hrs.	Pink	Achromic	Achromic	Achromic

The sacs were tested before and after dialysis, and were found free from leaks.

Taka diastase therefore dialyzes, though slowly, through the collodion sac.

FILTRATION THROUGH THE COLLODION SAC.

The following table gives the results of several filtrations of taka diastase through thin collodion sacs, under conditions similar to those given in connection with ptyalin. The taka diastase was employed in a 1:1,000 solution in 0.7 per cent. NaCl. The solution was passed through a single thickness of ordinary filter paper before collodion filtration. Controls were made in each instance from the unfiltered liquid remaining in sac after filtration. The controls and filtrates were tested by adding 1 c.c. of the liquid to 30 c.c. of a 1 per cent. starch solution and digesting in water bath at 37°. A blank control was made in each instance with 30 c.c. of the starch solution. The sacs were tested at a pressure of three inches and were found to be free from leaks before filtration.

TABLE XX.

No.	Filt. Pressure	Time of Filt.	Am't Filtered	Color Reaction with Iodine of		
				Starch Control	Diastase Control	Filtrate
	(Ins.)	(Hrs.)	(c.c.)			
1.....	2½–3½	2	3.8	Blue in 19 hrs.	Achr. in 2–2½ hrs.	Purple in 6 hrs. Achr. in 19 hrs.
2.....	2–3	2	4.2	Blue in 17 hrs.	Achr. in *ca.* 3 hrs.	Purple in 3½ hrs. Achr. in 17 hrs.
3.....	2–3½	1½	3.5	Blue in 24 hrs.	Achr. in 1 hr. 40 min.–2 hrs. 50 min.	Achr. in 22–24 hrs.
4.....	3⅛	1½	5.0	Blue in 24 hrs.	Achr. in *ca.* 3 hrs.	Achr. in 18–24 hrs.

The sacs used in filtrations 2, 3, and 4 were tested after filtration and found to be free from leakage. The sac used in filtration 1 showed leakage at 2½ inches.

The sac used in filtration of No 2, after thorough washing, was filled with 5 c.c. of the 1 : 1,000 diastase solution and immersed in 20 c.c. of a 0.01 per cent. starch solution. In another sac were placed 5 c.c. of the same solution, previously boiled, and this was immersed similarly, and served as a control. Both sacs were placed in the incubator at 37°. A control or initial test of the starch solution gave a blue reaction with iodine. At the end of one and one-half hours 2 c.c. of the starch solution were removed from each tube and tested with iodine. The starch in the control tube gave a deep-blue color, whereas that in the tube containing sac No. 2 gave an achromic reaction.

The sac, after the dialysis, was tested at 3 inches' pressure and was found to be free from leakage.

It will be seen from the experiment that, although the sac greatly retarded the passage of taka diastase, by filtration, it permitted a ready dialysis of the enzyme.

It has been shown that ptyalin is totally retained by the collodion filter, but taka diastase passes through to a considerable extent. It seemed possible that the retention of ptyalin was due to the presence of mucin, and if such was the case, a similar behavior could be expected of taka diastase under like conditions. The following experiments were made in order to show the effect of mucin:

A quantity of saliva which had been filtered through a single thickness of ordinary filter paper was boiled for five minutes in order to destroy the ptyalin. After cooling, 10 c.c. of this saliva were added to 10 c.c. of a 1 per cent. taka diastase solution and thoroughly mixed. The mixture was filtered through a collodion sac in the manner previously described. The filtrate and the control were tested by adding 1 c.c. of the liquid to 20 c.c. of the 1 per cent. starch solution. Blank controls were made as before. The following table gives the results of two such filtrations:

TABLE XXI.

No.	Filt. Pressure	Time of Filt.	Am't. Filtered	Iodine Reaction of		
				Starch Control	Diastase Control	Filtrate
	(Ins.)	(Hrs.)	(c.c.)			
1.....	3–3¼	1½	3.3	Blue in 20½ h.	Achr. in 6 hrs. +	{ Purple in 6 hrs. / Achr. in 20½ hrs.
2.....	3¼	1½	3.8	Blue in 20 hrs.	{ Purple in 3 hrs. / Achr. in 17½ hrs.	{ Deep purple in 3 hrs. / Red in 20 hrs.

At the close of the experiments the sacs were tested at a pressure of 3¾ and 5 inches respectively, and were found to be free from leakage.

The preceding filtrations were with mixtures of boiled saliva and taka

diastase. The two following experiments were made with raw saliva and 1 per cent. taka diastase in equal parts. Inasmuch as ptyalin does not filter through the collodion sac, a positive result must be due to the passage of taka diastase, notwithstanding the presence of mucin and other saliva constituents.

EXPERIMENT I.

Three cubic centimeters of the mixture filtered through a sac in 1 hour at a pressure of 2½–3½ inches. The sac was tested after filtration and showed no leak at 5 + inches.

Two cubic centimeters of this filtrate added to 20 c.c. of a 1 per cent. starch solution and left at 36° gave a purple test in 5 hours and an achromic reaction in 21 hours.

The blank control (20 c.c. of a 1 per cent. starch solution left at 36°) gave a blue reaction at the end of 21 hours.

The activity of the mixture in the sac was tested by adding 2 c.c. to 20 c.c. of a 1 per cent. starch solution and left at 36°. An achromic reaction was obtained in 7 minutes.

EXPERIMENT II.

Three cubic centimeters of the mixture filtered through a sac in 1 hour at a pressure of 2¼–3¼ inches. No leak could be detected in the sac after filtration, even at 6 inches' pressure.

Two cubic centimeters of this filtrate were added to 20 c.c. of the starch solution and left at 36°. An achromic reaction was obtained in 32 to 42 minutes.

The blank control (20 c.c. starch solution at 36°) gave a deep purple at the end of 42 minutes.

Two cubic centimeters of the mixture remaining in the sac were added to 20 c.c. of the starch solution at 36°. Iodine gave an achromic reaction in 40 minutes.

These experiments show conclusively that the presence of mucin does not prevent the passage of taka diastase through the collodion filter. It is probably also without effect on the ptyalin molecule.

Summing up, it will be seen that taka diastase is not removed from its solution by filtration through paper; it passes readily through the Berkefield filter, partially through the collodion filter, and dialyzes through the collodion membrane. It is injuriously affected by aeration.

RENNET.

EXPERIMENT I.

Paper filtration.—That rennet is removed from its solution by filtration through hardened filter paper is evidenced by the following results:

The rennet solution used in this experiment was prepared by dissolving one tablet of the commercial preparation (Chr. Hansen) in 100 c.c. of physiological salt (0.7 per cent.) solution; the resulting solution was then filtered through a single thickness of ordinary filter paper. This clear solution was not used directly, but served for the preparation of a 1:40,000 dilution. In each experiment 200 c.c. of the 1:40,000 rennet solution, in physiological salt, were used. Of this amount 195 c.c. served for the purpose of filtration, and 5 c.c. for the control tests. The rennet activity of each filtrate was tested by adding 1 c.c. of each to 5 c.c. of fresh milk in a test-tube kept at about 37° in a water-bath and observing the time required for coagulation. The rennet experiments covered a number of days, and fresh dairy milk was obtained each morning for that day's work. The filtering liquids as well as the controls were kept under black cloth. A common control was used for the "6×2" and "8×2" filtrates. The filter paper employed was ordinary hardened filter paper cut into squares of 15 cm. each.

TABLE XXII.

1 C.C. OF FILTERED 1:40,000 RENNET SOLUTION + 5 C.C. MILK AT 36°.

No.	Duration of Filtration	Digestion Temp.	Time Required to Produce Coagulation by						
			"2×2"		"4×2"		"6×2"		"8×2"
			Control	Filtrate	Control	Filtrate	Control	Filtrate	Filtrate
	Min.		Min.	Min.	Min.	Min.	Min.		
1...	110	36°	58	85–135	45–55	238	52	5 hrs. 15 min.	Neg. 18 hrs.
2...	130	34–37°	26–32	52	27	94–123	25	Slight Coag. 16 hrs.	Slight Coag, 16 hrs.
3...	170	37°	90	152–197	94–109	110–155	73–103	5–6 hrs	5–16 hrs.

The experiments tabulated above clearly show that successive filtrations of a rennet solution rapidly decrease its activity. In order to determine whether or not the removal of the enzyme was due to fixation, aeration over crumpled pieces of filter paper was resorted to as in the case of the ptyalin experiments already described.

EXPERIMENT II.

Effect of aeration.— In these experiments the liquid was aerated in the special tube mentioned heretofore, over bits of filter paper, glass beads, and garnets. It was found that after three hours' aeration, either over crumpled paper bits or over glass beads, the liquid within the tube was without any coagulating action. The fact that the glass beads exerted the same action as the bits of paper indicated that the enzyme was destroyed and not merely fixed or retained by the paper. Moreover, all attempts at extraction with glycerin of enzyme possibly fixed by the paper bits produced negative results.

In order to make sure that the rapid destruction of the enzyme was not due to the alkalinity resulting from the action of the water on the walls of the tubes, on the beads themselves, or on

the plug of glass-wool employed in keeping the paper bits in the tubes, the experiments were repeated with aeration tubes of Bohemian glass. One of these was filled with thoroughly cleansed garnets in place of the beads and the glass-wool. A second tube was filled with crumpled bits of paper, while as a further check, a third aeration tube was set up as a control in which the liquid was aerated alone, that is, not over garnets or paper.

Under these conditions the same ready destruction of the enzyme was obtained. In the control aeration tube the destruction was less rapid, six or more hours being required to destroy totally the enzyme, whereas over paper or garnets the rennet was destroyed in two to three hours. Control tests made with the unaerated rennet solution showed unimpaired activity during the time of these experiments. Moreover, it should be stated that tests were made with phenolphthalein of all liquids after aeration and showed an absence of alkalinity.

These facts then indicate that rennet in solution is readily destroyed by aeration, which destruction is hastened by the surface action of paper bits or garnets.

Effect of Berkefeld filters.—The retention of enzymes by porous porcelain filters has been observed in numerous instances. Gautier[16] called attention to the fact that, in the filtration of gastric juice through the porcelain bougie the pepsin was largely retained on the filter. Fermi and Pernossi[17] later experimented on the passage of trypsin and pepsin through the Chamberland bougie. They filtered a 1:500 solution of trypsin, containing five per cent. NaCl, through the bougie at a pressure of two atmospheres, and found a decrease of about one-half in the activity of the filtrate as compared with that of the original solution. The filtration of a 1:200 solution, containing no NaCl, caused a loss of about one-fourth of its strength, while the filtration of a 1:200 solution containing two per cent. of Na_2CO_3 resulted in the loss of about half of the strength of the solution.

In order to show that the decrease in activity was not due to the removal of undissolved trypsin which might be present, they filtered a 1:200 solution of the enzyme through the bougie at one atmosphere pressure. This filtrate was again filtered through

the bougie, and the resulting filtrate was passed through the filter once more, giving thus a triple filtration of the solution. They tested their controls and the filtrates by addition to gelatin, and found that the successive filtrates progressively decreased in activity. This fact was taken to prove that the weakening of the filtrate was not due to the retention of undissolved particles.

Setting aside the possibility that the trypsin could be destroyed by the filtration process, they reached the conclusion that the loss of the activity of the filtered solution was due to the retention of the molecules by the filter. To quote their words:

"We come to the conclusion that trypsin passes through the porcelain filter in such a way that, when one repeats (four to five times) the filtration of the same solution, the trypsin remains entirely on the filter."

They also observed a weakening in the activity of solutions of pepsin by refiltering through the porcelain bougie.

Achalme[18] also noted the retention of pancreatin by the bougie, but according to his statement the pancreatin loses only about 25–35 per cent. of its diastatic power, when filtered at a temperature of 35–38°.

Lea (*loc. cit.*) observed the retention of the urea ferment by the porous battery cell. Miquel (*loc. cit.*) was for a long time unable to pass urase through the porcelain bougie, but finally succeeded in obtaining a passage of the ferment after displacing the atmospheric air in the pores of the filter with illuminating gas.

Fermi[19] observed the total retention by the porcelain filter of the inverting enzyme of B. megaterium.

Buchner[20] succeeded in obtaining a partial passage of zymase through both the Berkefeld and Chamberland bougies. Fernbach[21] has shown that the sucrase of Aspergillus niger is totally retained by the porcelain bougie, whereas the sucrases of the Champagne yeast, of Saccharomyces Pastorianus, and of pale ale pass *en totalité* through the filter. According to Effront,[22] the invertin of yeast passes the Chamberland filter, whereas that of aspergillus does not. Ehrlich and Morgenroth[23] have pointed out that the complement in goat's blood causing the solution of rabbit corpuscles is retained on the Pukallfilter.

Vedder,[24] in working on the bacteriolytic complements in the

rabbit's blood, filtered the serum through Chamberland and Berkefeld filters, with the following results:

> The complements for all the organisms tested (B. typhosus, B. coli communis, B. dysenteriæ, and S. pyogenes aureus) may be separated from the serum by filtration through a Chamberland filter: but by passage through a Berkefeld filter the complements for certain organisms are retained (B. coli communis, S. pyog. aureus, and B. dysenteriæ, partially), while those for others (B. typhosus, B. dysenteriæ partially) pass through — a further indication of the multiplicity of the bacteriolytic complement. By filtration through a Chamberland filter it is possible to separate the agglutinins from the bactericidal substances of a serum.

We have known but little hitherto about the filtration of rennet. According to Oppenheimer,[25] Lea[26] found that vegetable as well as animal rennet was retained by the kaolin filter. In the reference cited by Oppenheimer we find no authority for such a statement. Lea found a milk-coagulating ferment in the plant Withania coagulans. In order to decolorize its solution he added finely divided kaolin, which addition resulted in the weakening of the enzyme. No mention is made of filtration through kaolin. In view of a more recent paper by Lea (and Dickinson),[27] in reply to Fick's theory as to the action of rennet, it seems improbable that he has witnessed any such retention. We quote the following experiments:

> Some milk was placed in a narrow (2 cm. wide) porous battery cell made of extremely thin earthenware. The cell was then immersed in a beaker of rennin solution, so that the level was the same inside and outside the cell, and digested at 40° for seventeen hours. At the end of this time there was not a trace of clot in the milk or on the inner surface of the cell. The experiment was repeated for a period of twenty-seven hours, with the same result. It appeared to us that if Fick's view is correct, then a clot might have been expected to form in the above experiment, if not throughout the whole mass of the milk, at least along the inner walls of the cell: but no trace of a clot was observed. It would perhaps, however, be unwise to lay too much stress on this experiment, for we know but little of what is happening in the pores of the cell's wall when it is used as a septum between two different fluids. But it is scarcely conceivable that the rennin should not have come in contact with the milk, bearing in mind that such a cell is permeated by pores through which a filtration of fluids can be carried on under pressure.

Lea and Dickinson did not attempt, however, to filter rennet solution through these pores under pressure.

Roberts[28] (1880) found that the brine extract of pancreas

when filtered under vacuum pressure through porous earthenware possessed an undiminished faculty for curdling milk, but it had almost no power of dissolving the curds. The curdling ferment had evidently traversed the earthenware freely, but only traces of trypsin had passed through.

Conn[29] (1892) separated rennet from the proteolytic enzyme of certain bacilli (B. mesentericus vulgatus?) by mixing the products of the organisms with milk and allowing coagulation to occur. This coagulum was broken up, then well shaken with distilled water, and the resulting solution filtered through porcelain. H_2SO_4 was added until the liquid contained 0.1 per cent., and NaCl was then added to saturation. A white granular-looking scum floated to the top, which was found to be almost pure rennet. The proteolytic enzyme remained behind in the brine.

Korschun[30] recently published a paper from which it appears that he has filtered rennet solution through both Berkefeld and Chamberland bougies. He gives no description of his process except that he used a little Berkefeld bougie. He finds a partial, but not complete, retention of the coagulating enzyme by the filter.

We know but little of the filter employed by Roberts. If, however, his results are correct, they give us an important distinction between pancreatic and gastric rennet; the passage of the rennet employed by Conn may be due to the fact that it is of vegetable origin. As regards Korschun's work, we are inclined to believe that he used imperfect bougies, or a rennet differing in properties from that employed by us. Our own experiments will show that the Berkefeld bougie, in perfect condition, will deny passage to the enzyme rennet, whether the filtration force be simply gravity, or 100 pounds' pressure to the square inch. On the other hand, old used bougies become more or less permeable.

EXPERIMENT III.

In this, as in all succeeding experiments, the rennet solution was filtered, either in the dilute form in which it was used, or previous to dilution, through one thickness of ordinary thin filter paper to insure absence of undissolved particles of rennet. The rennet was either that of Chr. Hansen or of Parke, Davis & Co., which commercial preparations were found to be identical in strength and behavior. Seven-tenths per cent. NaCl was used as the solvent for these solutions. The bougies were sterilized in every instance before each

individual filtration. It was observed that new Berkefeld bougies contain a considerable quantity of alkali, which passes into solution in the liquids traversing the bougie. Hence, before each individual filtration the bougie was washed by drawing through it hot distilled water, until the wash-water failed to give an alkaline reaction to phenolphthalein. The bougie was then cooled down to room temperature by passage of cold distilled water. Every enzyme filtrate was tested and found to be alkali-free. In the high-pressure filtration experiments the bougies were further washed by forcing distilled water through them at the pressure employed in the subsequent rennet filtration. The filtering apparatus employed was that described by Novy.[31]

Filtration by gravity.—One hundred and eighty cubic centimeters of a 1:10,000 rennet solution filtered through a Berkefeld bougie at gravity pressure in two hours. A portion of the solution was previously set aside to serve as control. The filtrate and control were tested by adding 1 c.c. of each to portions of 5 c.c. of milk. Blank controls of milk were made to indicate coagulating action, if any, of micro-organisms. The digestion temperature was 36°.

The tests which were made in this experiment resulted as follows:

1 c.c. of control enzyme + 5 c.c. milk gave coagulation in 23 min.
1 c.c. filtrate + 5 c.c. milk gave no coagulation in 6 hrs.
Milk control gave no coagulation in 6 hrs.

It appears, therefore, that Rennet does not pass through the Berkefeld bougie in gravity filtration.

EXPERIMENT IV.

Filtration at vacuum pressure.—The following tabulated set of filtrations was carried on by aspiration. In the first three filtrations the liquid was allowed to pass through the bougie immediately upon beginning of suction, the pressure increasing during the filtration process to the point indicated in the table. In the latter three filtrations the receiving flask was exhausted before admission of the liquid, so that the entire filtration was carried on at the pressure indicated in the table. Milk controls indicated in the table are blank controls consisting of merely 5 c.c. of milk, without addition of enzyme. The tests of controls and filtrates were made by adding 1 c.c. of the liquid tested to 5 c.c. of milk.

TABLE XXIII.

No.	Dilution	Pressure	Duration of Filtration	Amt. Filt.	Temp. of Digestion	Coagulating Action of		
						Control		Filtrate
						Milk	Rennet	
		Ins.	Min. Sec.	c.c.				
1.....	1:20,000	26⅜	1 50	195	37.5–36	.....	Coag., 40–44 m.	Neg. in 20 hrs.
2.....	1:10,000	27½	1 30	195	40°5	.....	Coag. in 20 m.	Neg. in 8 hrs.
3.....	1:10,000	27½	0 62	196	39	.....	Coag. in 21 m.	Neg. in 2 hrs.
4.....	1:10,000	27⅝	1 30	195	36	Neg., 2 h. 5 m.	Coag. in 19 m.	Neg., 2 h. 5 m.
5.....	1:10,000	26⅛	0 75	195	35	Neg. in 11 hrs.	Coag. in 21 m.	Neg. in 11 hrs.
6.....	1:10,000	27⅝	0 85	195	35	Neg. in 7 hrs.	Coag. in 23 m.	Neg. in 7 hrs.

As above mentioned, Achalme found that pancreatin loses only 25–35 per cent. of its activity when filtered through the porcelain bougie, provided the solution is heated to 35–38°. In the following filtrations not only were the solutions warmed to the temperature indicated in the table, but the filters were also heated to the same temperature, respectively, by the repeated passage of warmed distilled water through the bougie. Thus, in the following filtrations the solutions of 37, 43, and 46° passed through filters of the same respective temperatures. To determine the effect, if any, on the enzyme of warming, controls were made with the liquid both at room temperature and at filtration temperature.

TABLE XXIV.

No.	Dilution	Pressure	Time of Filtration	Amt. Filt.	Temp. of Filtration	Temp. of Digestion	Coagulating Action of		
							Control		Filtrate
							Room Temp.	Filt. Temp.	
		in.	sec.	c.c.			Coag. in	Coag. in	Negative in
1....	1 : 10,000	28	65	190	37°	37–35	21 min.	21 min.	3 hrs. 50 min.
2....	1 : 10,000	27¾	....	190	43	39–37	20 min.	20 min.	16 hrs.
3....	1 : 20,000	26¾	110	190	46	37	46 min.	46 min.	4 hrs. 30 min.

An attempt made to filter at 52° with its corresponding increase in molecular motility, however, resulted in the destruction of the enzyme. In the four filtrations the liquids were gently warmed over the Bunsen flame, and then filtered immediately upon arriving at the desired temperature.

We have already called attention to the fact that Miquel succeeded in filtering urase through the porcelain bougie in an atmosphere of illuminating gas, whereas the enzyme failed to traverse the bougie in the presence of air. The following experiment is designed to demonstrate the behavior of rennet as regards filtration in an atmosphere of inert gas:

a) One hundred and ninety-five cubic centimeters of 1 : 10,000 rennet solution were filtered through the Berkefeld bougie at 28 inches' pressure in 45 seconds in an atmosphere of air. Controls and filtrates were tested as before.

1 c.c. control + 5 c.c. milk at 36°—coagulated in 20 min.
1 c.c. filtrate + 5 c.c. milk at 36°—coagulated in about 5 hrs.
Milk control negative in 5 hrs.

b) Scrubbed and sterilized the bougie used in *a*. Into the mouth of the filter globe inserted a rubber stopper with two apertures, one of which carried a tube connected with the illuminating-gas supply, the other carrying the tube of a separatory funnel. Closed the cock of the funnel and poured into the latter 190 c.c. of 1 : 10,000 rennet solution. Exhaustion was produced in the globe, bougie, and receiver, and gas was then admitted into the apparatus until the pressure fell to about five inches. Exhaustion was again produced, and gas was readmitted until the pressure fell to about five inches. The rubber connection between the receiver and the cylinder was then clamped, and the receiver exhausted, gas in the meantime continuously passing into the globe and bougie. When the receiver was exhausted, the clamp was released, and at the same time the enzyme solution was allowed to pass from the separatory funnel into the globe, leaving behind enough liquid in the funnel, however, to prevent entrance of air into the globe. This remaining liquid was also used for the control test. Liquid thus passed through the bougie in an atmosphere of illuminating gas, and in the absence of air.

Pressure, 27⅞ inches; time of filtration, 60 seconds; amount filtered, 185 c.c.

1 c.c. control + 5 c.c. milk at 36 degrees—coagulated in 23 min.
1 c.c. filtrate + 5 c.c. milk at 36 degrees—coagulated in 6–7 hrs.
Milk control—coagulated in 6–7 hrs.

c) Scrubbed and sterilized the bougie and washed the same thoroughly. Then filtered 195 c.c. of 1 : 10,000 rennet solution in an atmosphere of air at a pressure of 28⅛ inches in sixty seconds.

1 c.c. control + 5 c.c. milk 36° —coagulated in 15 min.
1 c.c. filtrate + 5 c.c. milk 36° —coagulated in 6–7 hrs.
Milk control—coagulated in 6–7 hrs.

It does not seem, after comparison with filtrations *b* and *c*, that any of the enzyme passed through the bougie in filtration *a*. The milk used in this experiment was undoubtedly very rich in organisms, and had we kept the milk control under observation a few minutes longer, we should no doubt have observed coagulation. As before mentioned, milk was obtained fresh each morning for the work of the ensuing day, but it seems probable that on this particular day an aged milk was substituted.

Rennet, therefore, does not pass through the Berkefeld bougie in an atmosphere of illuminating gas. Is gas absolutely inert toward rennet, or is the absence of activity in the filtrate due to the destruction of the enzyme by the gas? The following experiment was made in order to answer this question:

In each of three test-tubes of Bohemian glass, each provided with a side arm, were placed 20 c.c. of a 1 : 10,000 rennet solution. The tubes were designated *A*, *B*, and *C*, respectively.

Through rennet in *A* gas was passed for five minutes: the rennet in *B* was aerated for five minutes, entrance of foreign particles into the tube being guarded against by a cotton plug in the side arm: the contents of *C* served as control. Each of these was tested, with the following results:

1 c.c. of control + 5 c.c. milk at 36°—coagulated in 23 min.
1 c.c. of *A* + 5 c.c. milk at 36°—coagulated in 21 min.
1 c c. of *B* + 5 c.c. milk at 36°—coagulated in 21 min.
Milk control was negative.

Illuminating gas can therefore be said to be without effect on rennet solutions.

The foregoing experiments show that rennet solutions do not pass through the Berkefeld bougie at vacuum pressure, either at room temperature or at higher temperature, or in an atmosphere of illuminating gas.

EXPERIMENT V.

Filtration at fifty pounds' pressure.—The procedure of pressure filtration employed in the following experiment is that described by Novy (*loc. cit.*). Save in the first two filtrations, all filtered liquids were forced directly into the spherical receiver described in the text, thereby obviating the effect on the filtrate of possible leakage in the apparatus.

A. One-hundred and ninety-five cubic centimeters of a 1:10,000 rennet solution were subjected to filtration at 50 pounds' pressure through the Berkefeld bougie. The time of passage was about fifteen seconds. The tests of control and filtrate resulted as follows:

1 c.c. control + 5 c.c. milk at 34–35°—coagulated in 18 min.
1 c.c. filtrate + 5 c.c. milk at 34–35°—coagulated in 9–11½ hrs.

No milk control was made, but it is evident that no enzyme passed through the bougie.

The bougie was employed in the meantime in a filtration test at 100 pounds' pressure (Experiment VI*a*), after which it was used for another filtration at 50 pounds. One hundred and ninety-five cubic centimeters of the solution were filtered in about ten seconds.

1 c.c. control + 5 c.c. milk at 36°—coagulated in 23 min.
1 c.c. filtrate + 5 c.c. milk at 36°—coagulated in 40 min.
No milk control.

In the first filtration the bougie had previously been used for nothing but vacuum-pressure filtration, and no enzyme passed through the bougie at 50 pounds' pressure. An ensuing filtration at still higher pressure had evidently worn channels in the filter, and a second filtration at 50 pounds caused a retention of less than half of the enzyme.

B. In this test a new bougie was employed. One hundred and ninety cubic centimeters of a 1:10,000 rennet solution were filtered in eight seconds at 50 pounds' pressure.

1 c.c. control + 5 c.c. milk at 36°—coagulated in 24 min.
1 c.c. filtrate + 5 c.c. milk at 36°—negative in 2 hrs. 15 min.
Milk control—negative in 2 hrs. 15 min.

Observations not taken after two and a quarter hours.

In the meantime the bougie was used for four filtrations at 100 pounds' pressure. Then another filtration was made at 50 pounds. One hundred and ninety five c.c. were filtered in ten seconds. The tests resulted as follows:

1 c.c. control + 5 c.c. milk at 35° — coagulated in 21 min.
1 c.c. filtrate + 5 c.c. milk at 35° — coagulated in 32 min.
The milk control was negative.

One hundred and ninety-five cubic centimeters of a 1 : 10,000 rennet solution, made up from the same 1 : 1,000 solution as that used in the preceding filtration, were filtered through another bougie (not new) at 50 pounds' pressure in ten seconds:

1 c.c. control + 5 c.c. milk at 34.5° — coagulated in 22 min.
1 c.c. filtrate + 5 c.c. milk at 34.5° — coagulated in 2 hrs. 20 min.
The milk control was negative.

The preceding experiments go to show that repeated filtrations under high pressure through the same bougie cause a decrease in the retaining properties of the bougie. The following series of filtrations was designed to bring out this point more fully.

C. Six filtrations of a 1 : 10,000 rennet solution were made through the same bougie at 50 pounds' pressure. The bougie was sterilized before each filtration by heating in an autoclave in about two liters of distilled water to 136-140° C. Twelve hundred and twenty cubic centimeters of a 1 : 10,000 rennet solution were made up and filtered in the six portions indicated below. The tests of filtrates and control were made as in the preceding experiments Milk controls, without the addition of rennet, were also made as before. The digestion temperature was 35°.

TABLE XXV.

No.	Amount of Filtrates	Time of Filtration	Coagulating Action of		
			Control		Filtrate
			Milk	Rennet	
1.....	195 c.c.	9 sec.	Neg.	Coag. in 20 min.	Coag. in 1 hr. 45 min.
2. ...	195 c.c.	10 sec.	Neg.	Coag. in 21 min.	Coag. in 2 hrs. 54 min.
3.....	190 c.c.	15 sec.	Neg.	Coag. in 21 min.	Coag. in 1 hr. 55 min.
4.....	190 c.c.	14 sec.	Neg.	Coag. in 21 min.	Coag. in 3 hrs. 5 min.
5.....	150 c.c.	12 sec.	Neg.	Coag. in 21 min.	Coag. in 5 h. 53m.–6h. 8m.
6.....	130 c.c.	8 sec.	Neg.	Coag. in 22 min.	Neg. in 7 hrs. 15 min.

These results were rather surprising in that they seemed to indicate a clogging up of the pores of the filter by the rennet molecules and other substances, instead of the opening up of fresh channels. It will be observed, however, that in the former experiments which led to this series the intermediate filtrations were at 100 pounds' pressure, and not at 50 pounds.

To determine the effect of the concentration of the solution upon the retention of the enzyme by the pores of the filter, the following experiment was performed. It tends to indicate that concentration is without effect in retarding the passage. If anything, it shows that the more concentrated the solution the more readily will the enzyme pass through.

D. Made up 250 c.c. of a 1 : 1,000 rennet solution and diluted 10 c.c. of this to 200 c.c. with 0.7 per cent. NaCl. Filtered 195 c.c. of the resulting 1 : 20,000 solution through the bougie used in *C*, at 50 pounds' pressure. Sterilized the bougie and 195 c.c. of the 1 : 1,000 solution were filtered through it at 50 pounds' pressure. Tests of filtrates and controls were made as before. The digestion temperature was 35°.

TABLE XXVI.

Dilution	Time of Filtration	Coagulating Action of		
		Control		Filtrate
		Milk	Rennet	
1 : 20,000.....	11 sec.	Negative	Coag. in 34 min.	Coag. in 5½–7¼ hrs.
1 : 1,000.....	11 sec.	Negative	Coag. in 2 min.	Coag. in 13 min.

Thus the passage through the bougie of rennet solutions at 50 pounds' pressure depends entirely upon the bougie employed. The enzyme is totally retained by a new bougie. When old bougies are used, the enzyme passes through in varying amounts, depending upon the previous use to which the filter has been put, and also upon the concentration of the enzyme solution.

EXPERIMENT VI.

Filtration at 100 pounds' pressure.—a) One hundred and ninety-five cubic centimeters of a 1 : 10,000 rennet solution were filtered through the Berkefeld bougie used in Experiment *A* of the 50-pound pressure series. The liquid passed through in seven seconds.

1 c.c. of control + 5 c.c. milk at 39°—coagulated in 16 min.
1 c.c. of filtrate + 5 c.c. milk at 39°—coagulated in 1½–1⅔ hrs.
No milk control was made.

This showed that there was a partial retention of the enzyme by the filter. The bougie was washed by drawing through it about two liters of distilled water. It was then immersed in 50 c.c. of milk in a large test-tube, which was placed in an incubator at 37°. Beside it was placed another test-tube containing 50 c.c. of milk to serve as a control.

The milk in the tube containing the bougie curdled at the end of six and one-half hours, whereas the control milk was still perfectly liquid. During the succeeding nine hours coagulation occurred also in the control tube, but at the end of that time the milk in the bougie tube showed a much more marked coagulation. The curd was solid and a clear transparent whey was present, whereas that of the control was creamy.

This experiment goes to show that the lessened activity of the filtrate is not due to the destruction of the enzyme, but that this is probably retained in an active condition in the pores of the filter.

b) In this test a new bougie was employed. The filtration of 195 c.c. at 100 pounds' pressure was not complete because the stopper blew out of the globe, but not, however, before 70 c.c. of the liquid had passed through. This passage was practically instantaneous.

1 c.c. control + 5 c.c. milk at 36° — coagulated in 17 min.
1 c.c. filtrate + 5 c.c. milk at 36° — negative in 7½ hrs.
No milk control was made.

This test shows a complete retention of the enzyme by the filter.

c) Through this same bougie the following series of filtrations at 100 pounds' pressure was carried on. After each filtration the bougie was washed and then immersed in 50 c.c. of milk. Duclaux[32] states that the Chamberland bougie immersed in milk will cause coagulation by surface action, layers of coagulum due to the rupture of the equilibrium of the milk particles by surface action being deposited on the bougie. In the following experiments, as in *a*, the action of the bougie was controlled by a sterilized Berkefeld bougie, similarly immersed.

TABLE XXVII.

(Digestion temperature, 36 .)

No.	Amt. of Filtrate	Time of Filtration	Coagulating Action of			Coagulating Action of Bougie	
			Control		Filtrate		
			Milk	Rennet		Control	Filter
1.....	190 c.c.	5 sec.	Negative	Coagulated in 14 min.	Coagulated in 5 hrs.	Negative	Coagulated in 5 hrs. 57 min.
2.....	194 c.c.	5–10 sec.	Negative	Coagulated in 21 min.	Coagulated in 1½ hrs.	Negative	Coagulated in 8 hrs. 30 min.
3	195 c.c.	5–10 sec.	Negative	Coagulated in 26 min.	Coagulated in 43 min.	Negative	Coagulated in 8 hrs. 30 min.

It is thus seen that rennet is retained by a new Berkefeld filter even when filtered under a pressure of 100 pounds, but successive filtrations through the same bougie render it more permeable.

EXPERIMENT VII.

A new Berkefeld bougie was rendered alkali-free, as before indicated. It was then used for a series of nine filtrations of a 1 : 10,000 rennet solution. These were made in three sets: the first set consisted of three filtrations at vacuum pressure, the second of a like number at 50 pounds' pressure, and the third at 100 pounds' pressure. For the first two sets two liters of a 1 : 10,000 rennet solution were made up. For the third set, performed on the next day, a liter of a fresh 1 : 10,000 solution was prepared. In each instance 195 c.c. were filtered, and 5 c.c. were set aside for control. The controls were subjected to the same light and temperature conditions as the filtering liquid. The control and the corresponding filtrate were tested at the same time. The tests were made as before at 35°, by adding 1 c.c. of the liquid to 5 c.c. of milk. Blank controls of the milk were made as before. *In no instance did the enzyme traverse the filter.*

TABLE XXVIII.

No.	Pressure	Time of Filtration	Coagulating Action of		
			Control		Filtrate
			Milk	Rennet	
1.....	27 in.	75 sec.	Negative	Coagulated in 18 min.	Negative in 13 hrs.
2.....	27 in.	75 sec,	Negative	Coagulated in 18 min.	Negative in 12 hrs.
3.....	27 in.	90 sec.	Negative	Coagulated in 18 min.	Negative in 9¼ hrs.
1.....	50 lbs.	16 sec.	Negative	Coagulated in 18 min.	Negative in 7¾ hrs.
2.....	50 lbs.	16 sec.	Negative	Coagulated in 17 min.	Negative in 6¼ hrs.
3.....	50 lbs.	17 sec.	Negative	Coagulated in 17 min.	Negative in 5¼ hrs.
1.....	100 lbs.	10–15 sec.	Negative	Coagulated in 17 min.	Negative in 11 hrs.
2.....	100 lbs.	*ca.* 10 sec.	Negative	Coagulated in 17 min.	Negative in 11 hrs.
3.....	100 lbs.	*ca.* 10 sec.	Negative	Coagulated in 17 min.	Negative in 9½ hrs.

When the 50-pound set was examined ten hours after the last observation indicated in the table, coagulation had occurred in both filtrate and in blank milk control tubes. The coagulum in the filtrate tubes did not resemble the rennet control coagulum, which formed, on standing a few hours, a thick curd, but resembled rather the creamy coagulum of the milk control.

At the end of twelve hours coagulation occurred simultaneously in both milk control and the filtrate tube of Filtrates 1 and 2 of the 100-pound set.

In every instance there is a total retention of rennet by the Berkefeld bougie at vacuum pressure, and, save in the case of old bougies, there is the same complete absence of activity in the filtrates of the 50-pound and 100-pound pressure filtrations.

The enzyme is not destroyed or altered by the bougie, but is merely retained mechanically by the pores of the filter. It is almost inconceivable that the enzyme, in an experiment such as *b* of the 100-pound filtrations, should become destroyed or chemi-

cally altered during the almost instantaneous passage of the liquid through the bougie.

That the enzyme is not destroyed is evident from the fact that the bougies used in the 100-pound pressure filtrations, when immersed in milk, readily cause coagulation. That the pressure itself is without effect on the enzyme is clear from the demonstration that the same retention is observed in gravity filtration as in that at 100-pound pressure.

This fact is further shown by the following: 10 c.c. of a 1 : 10,000 solution of rennet were placed in a Pfungst autoclave and subjected to a pressure of 100 pounds for one hour at room temperature. In the meantime, as a control, 10 c.c. of the same solution were kept in a black box. At the end of the hour the coagulating action of each liquid was tested at 36°, with the following results:

1 c.c. autoclave rennet + 5 c.c. milk — coagulated in 34 min.
1 c.c. control rennet + 5 c.c. milk — coagulated in 34 min.
A milk control was negative.

It follows therefore that pressure is without effect.

EXPERIMENT VIII.

The following experiment was made in order to determine the effect, if any, exerted by the material composing the bougie upon the dissolved rennet:

A Berkefeld bougie was ground up in mortar to a very fine powder, which was then sterilized. To the powdered bougie in a beaker 190 c.c. of a 1:10,000 rennet solution were added, and the mixture was agitated by means of a mechanical stirrer for five minutes. A portion of 10 c.c. of the 1 : 10,000 solution was set aside as a control under the same temperature and light conditions as obtained for the mixture.

After the stirring ceased, the suspended particles settled very slowly and evenly. At the end of five and one-half hours the mixture was still very turbid. Ten cubic centimeters of mixture were then centrifugated, as also was the control, for thirty minutes. With the still cloudy liquid the following tests were made at 36°:

1 c.c. mixture + 5 c.c. milk — coagulated in 61 min.
1 c.c. control + 5 c.c. milk — coagulated in 36 min.
The milk control was negative.

After again sterilizing the powder, this experiment was repeated. The mixture was stirred this time for ten minutes and allowed to settle for two and three-quarter hours, after which it was centrifugated for one hour. A control solution was kept under the same conditions. The mixture remained turbid as in the preceding trial. The tests were made at 36°:

1 c.c. mixture + 5 c.c. milk—coagulated in 57 min.
1 c.c. control + 5 c.c. milk—coagulated in 30 min.
The milk control was negative.

The decrease noted above in the activity of the rennet solution is no doubt due to the carrying down of the enzyme molecules by the sedimenting of the diatomaceous earth particles which make up the Berkefeld bougie.

EXPERIMENT IX.

It may be assumed that the behavior of the filter is due to the presence of some substance in the bougie which, when dissolved out by the rennet solution, inhibits the action of the enzyme.

If there is anything of an inhibiting nature in the undisintegrated bougie, this substance must clearly appear in the filtrate. The following tests show the filtrate to be perfectly free from any substance of an inhibitory nature:

One hundred and ninety-five cubic centimeters of a 1 : 10,000 rennet solution were filtered through a Berkefeld bougie at a pressure of 27⅞ inches in eighty-five seconds. Fifty cubic centimeters of filtrate were then added to 50 c.c. of unfiltered solution, and the mixture was subjected to mechanical stirring for ten minutes. Another portion of 50 c.c. of the unfiltered solution was set aside for control. The tests were then made at 35°:

1 c.c. mixture + 5 c.c. milk—coagulated in 46 min.
½ c.c. control + ½ c.c. distilled water + 5 c.c. milk—coagulated in 46 min.
1 c.c. control + 5 c.c. milk—coagulated in 23 min.
1 c.c. filtrate + 5 c.c. milk—negative in 7 hours.
The milk control was negative in 7 hours.

Repeated experiments produced the same results and confirmed the belief that the filtered liquid had no inhibitory action other than that of a mere diluent.

This fact was also shown in another way. One hundred cubic centimeters of the filtrate obtained in the above experiment were evaporated in a Roux vacuum desiccator at 37° to 10 c.c. The following mixtures were then made with a 1 : 10,000 rennet solution:

A = 5 c.c. evaporated filtrate + 5 c.c. rennet sol.
B = 5 c.c. distilled water + 5 c.c. rennet sol.
C = 1 c.c. evaporated filtrate + 5 c.c. rennet sol.
D = 1 c.c. distilled water + 5 c.c. rennet sol.

One cubic centimeter of each solution was then added to portions of 5 c.c. of milk, kept at 35°, with the following results:

1 c.c. A + 5 c.c. milk—coagulated in 58 min.
1 c.c. B + 5 c.c. milk—coagulated in 44 min.
1 c.c. C + 5 c.c. milk—coagulated in 34 min.
1 c.c. D + 5 c.c. milk—coagulated in 30 min.
The milk control was negative.

The controls *B* and *D* are not strictly comparable to the test mixtures since they were made with distilled water, whereas the evaporated filtrate, owing to the fact that the rennet solutions were made up in physiological salt, contained seven per cent. of NaCl. To show the effect of seven per cent. of NaCl on a 1 : 10,000 rennet solution, the following mixtures were made and tested at 35°, as before:

A = 5 c.c. 7 per cent. NaCl sol. + 5 c.c. rennet sol.
B = 5 c.c. distilled water + 5 c.c. rennet sol.
C = 1 c.c. 7 per cent. NaCl sol. + 5 c.c. rennet sol.
D = 1 c.c. distilled water + 5 c.c. rennet sol.

Tested as follows:

	First Test	Second Test
1 c.c. *A* + 5 c.c. milk	coagulated in 66 min.	coagulated in 67 min.
1 c.c. *B* + 5 c.c. milk	coagulated in 50 min.	coagulated in 51 min.
1 c.c. *C* + 5 c.c. milk	coagulated in 30 min.	coagulated in 32 min.
1 c.c. *D* + 5 c.c. milk	coagulated in 30 min.	coagulated in 30 min.

It is thus seen that the retarding action of the evaporated filtrate in this experiment is due to the presence of seven per cent. NaCl.*

EXPERIMENT X.

Filtration through Chamberland filter.—The series of filtrations tabulated below show that the rennet molecule is also unable to pass through the pores of the Pasteur filter, size *F*. The bougie before use was thoroughly cleansed, ignited, and sterilized by autoclaving before each individual filtration. For each filtration at vacuum pressure 190 c.c. of a 1 : 10,000 rennet solution were employed. The filtrations at 50 pounds' pressure were made with 195 c.c. The filtrates were all tested for alkalinity and found to be neutral in reaction. The controls and tests, at 36°, were made as in the case of the Berkefeld filtrations.

TABLE XXIX.

No.	Pressure	Time of Filtration	Coagulating Action of		
			Control		Filtrate
			Milk	Rennet	
1.....	27½–27⅞ in.	3½ min.	Negative	Coag. in 25 min	Neg. in 10¼ hrs.
2.....	27⅞ in.	3¾ min.	Negative	Coag. in 22 min.	Neg. in 9¾ hrs.
3.....	27½–28⅛ in.	4 min.	Negative	Coag. in 23 min.	Neg. in 7 hrs.
1.....	50 lbs.	80 sec.	Negative	Coag. in 20 min.	Neg. in 13 hrs. 10 min.
2.....	50 lbs.	70 sec.	Negative	Coag. in 20 min.	Neg. in 11 hrs. 30 min.
3.....	50 lbs.	75 sec.	Negative	Coag. in 14 min.	Neg. in 10 hrs.

* *Cf.* OPPENHEIMER, *Die Fermente, etc.*, p. 149.

EXPERIMENT XI.

Filtration through collodion sacs.—Filtration through collodion sacs (as previously described) resulted in the absolute retention of the enzyme by the filter.

The filtrates and controls were tested at 36° as in previous experiments.

TABLE XXX.

No.	Dilution	Pressure	Time of Filtration	Am't Filtered	Coagulating Action of		
					Control		Filtrate
					Milk	Rennet	
1.....	1:10,000	2-3 in.	2 hrs.	6½ c.c.	Negative	Coag. in 23 min.	Neg. in 6 hrs.
2.....	1:1,000	2½ in.	2½ hrs.	4 c.c.	Negative	Coag. in 2 min.	Neg. in 8½ hrs.
3.....	1:1,000	4 in.	1 hr.	4 c.c.	Negative	Coag. in 3 min.	Neg. in 9 hrs.

EXPERIMENT XII.

Dialysis through collodion sacs.— It will be seen from the following, which is one of several similar experiments, that rennet dialyzes readily through the collodion sac.

Seven very thin sacs were prepared by rolling the tube used in their preparation twice in thin collodion. These were designated *C*, 1, 2, 3, 4, 5, and 6. Five cubic centimeters of a 1:1,000 rennet solution, previously boiled for five minutes, were placed in the sac marked *C*, and served as a control. In each of sacs 1–6, inclusive, were placed 5 c.c of a fresh 1:1,000 solution. Each sac was then immersed in a test-tube on foot containing 25 c.c. of milk and placed in a nincubator at 36°. The first evidence that the enzyme dialyzed through the walls of the sac was seen in the formation of a coagulum which adhered in clumps to the outside of the sac. This "bushing" increased in extent until in time the entire contents of the tube coagulated. The results obtained were as follows:

Slight "bushing" in Nos. 1, 3, 5, and 6 in 6 hrs.
Marked "bushing" in Nos. 1, 2, 3, 4, and 5 in 9 hrs.
Slight "bushing" in No. 6 in 9 hrs.
No "bushing" in control in 9 hrs.

When next examined at the end of twenty-one hours, the control showed a soft, creamy coagulum extending through the mass of milk, whereas in Tubes 1–6, inclusive, the curd formed a solid layer at the bottom of the tube, while above it was a layer of clear liquid. Solid masses of curd were adherent to each sac.

The sacs employed in this experiment were tested before and after use, and were found to be free from leaks.

As in the case of ptyalin, dialysis was shown to occur through the same sacs, which did not allow filtration of the enzyme.

By way of contrast to the behavior of the collodion sac may be given an experiment wherein dialysis was attempted by placing within a Berkefeld bougie 10 c.c. of a 1:10,000 rennet. The bougie was placed in a test-tube in 40 c.c. of milk and set aside at 36°. No dialysis occurred; the result in this case agreeing with that of Lea and Dickinson (*vide supra*).

The foregoing study of the behavior of rennet shows (1) that it is removed from its solution by filtration through hardened filter paper; (2) that this removal is not due to fixation by the paper; (3) that it is readily destroyed by aeration and surface action; (4) that it is removed completely from its solution by the pores of a new Berkefeld bougie, even under high pressure; (5) that this removal is mechanical and is not due to chemical destruction; (6) that it is removed in like manner by the Chamberland filter; (7) that it readily dialyzes through the collodion sac, but not through a Berkefeld bougie; (8) that it is removed from its solution when filtered through a collodion sac.

PEPSIN.

PAPER FILTRATION.

The pepsin used in these experiments was that of Parke, Davis & Co. The solutions were prepared by dissolving the preparation in 0.7 per cent. NaCl. They were filtered before use, either before or after dilution, through a single thickness of ordinary filter paper. The action of filtrates, controls, etc., in these experiments was tested by adding 0.1 g. of fibrin to 5 c.c. of the pepsin solution, to which had been added 1 c.c. of a 10 per cent. HCl. This fibrin was obtained from hog's blood and was well washed first in running water, then in distilled water, and was carefully pressed, just before weighing, between several thicknesses of filter paper, in order to remove as much moisture as possible.

EXPERIMENT I.

The following table gives the results of three sets of filtrations of a 1:16,000 pepsin solution. A common control was used for the "6×2" and "8×2" filtrates. The arrangement of filters, it may be added, was the same as that heretofore employed.

TABLE XXXI.

No.	Duration of Filtration	Digestion Temperature	Time Required to Produce Solution of Fibrin by						
			"2×2"		"4×2"		"6×2"		"8×2"
			Control	Filtrate	Control	Filtrate	Control	Filtrate	Filtrate
1...	2 hrs.	37°	5 hrs.	5 hrs.+	3-4 hrs.	4-5 hrs.	3 hrs.	Neg. in 24 hrs.	Neg. in 24 hrs.
2...	2 hrs. 35 min.	38°	2-3 hrs.	3 hrs.+	2-3 hrs.	¾ dissol. in 18 hrs.	2-3 hrs.	Neg. in 18 hrs.	Neg. in 18 hrs.
3...	1 hr. 50 min.	35°	¾ dissol. in 4 hrs.	4 hrs.	½ dissol. in 4 hrs.	6-7 hrs.	¾ dissol. in 3 hrs.	¾ dissol. in 11 hrs.	¾ dissol. in 11 hrs.

A blank control was made in each case with 0.1 g. fibrin in 5 c.c. of 0.7 per cent. NaCl containing 0.1 c.c. of a 10 per cent. HCl. These were uniformly negative during the time of the experiment.

This experiment clearly shows that pepsin is removed from its solution by hardened filter paper.

To determine if this decrease in the activity of the enzyme solutions was due to fixation by the filter paper, experiments were performed similar to those demonstrating the fixation of ptyalin, but no fixation could be demonstrated. Aeration was observed to have a destructive effect upon the enzyme, and this effect was aided by the surface action of the paper bits or garnets over which the pepsin was aerated.

EXPERIMENT II.

The effect of Berkefeld filters.— The table below shows the effect of Berkefeld filtration on 1:16,000 pepsin solutions. One hundred and ninety cubic centimeters were filtered in each instance. The filtrate and control tests and blank controls were made as before. The digestion was carried on at 36–37°. The bougie was sterilized before each filtration by autoclaving.

TABLE XXXII.

No.	Pressure	Time of Filtration	Time Required to Produce Solution of Fibrin by		
			Control		Filtrate
			Blank	Pepsin	
1.....	27⅞ in.	65 sec.	Negative	1-2 hrs.	10-20 hrs.
2.....	27¾ in.	65 sec.	Negative	*ca.* 1½ hrs.	8-18 hrs.
3.....	27¾ in.	65 sec.	Negative	*ca.* 1½ hrs.	½ dissol. in 10 hrs.
4.....	50 lbs.	16 sec.	Negative	1½-2 hrs.	12 hrs. +
5.....	50 lbs.	16 sec.	Negative	*ca.* 2 hrs.	¾ dissol. in 18 hrs.
6.....	50 lbs.	20 sec.	Negative	*ca.* 2 hrs.	¾ dissol. in 18 hrs.
7.....	100 lbs.	5 sec.	Negative	*ca.* 2 hrs.	¾ dissol. in 24 hrs. +
8.....	100 lbs.	10 sec.	Negative	*ca.* 2 hrs.	¾ dissol. in 24 hrs.
9.....	100 lbs.	8 sec.	Negative	2-3 hrs.	¾ dissol. in 24 hrs.

In Tests 1–4, inclusive, hog fibrin several days old was used.

The two following filtrations were made with the same bougie used above. The tests were carried out at 36°.

TABLE XXXIII.

No.	Pressure	Time of Filtration	Solvent Action on Fibrin of		
			Control		Filtrate
			Blank	Pepsin	
1.....	28 in.	70 sec.	Neg.	Solution in 2–3 hrs.	Negative in 15 hrs.
2.....	27⅞ in.	70 sec.	Neg.	Solution in 2–3 hrs.	Negative in 14 hrs.

In Table XXXII a less ready passage is observed at 100 pounds' pressure than at vacuum pressure. The subsequent filtrations through the same bougie at vacuum pressure indicated a clogging of the pores of the filter by pepsin and other substances in the successive filtrations. Fermi and Pernossi (*loc. cit.*) call attention to the fact that the passage of pepsin through the Chamberland bougie is less ready when the bougie used had previously been employed for the filtration of colloidal substances.

These experiments go to show that a marked difference exists between rennet and pepsin in regard to the passage through the Berkefeld filter.

EXPERIMENT III.

Filtration through collodion sac.—In a series of trials it was shown that pepsin does not pass through the collodion filter. The results are given in the following table. The pepsin solutions employed were 1:16,000 and the digestion was carried on at 37°.

TABLE XXXIV.

No.	Pressure	Am't Filtered	Time of Filtration	Time Required to Dissolve Fibrin by		
				Control		Filtrate
				Blank	Pepsin	
1.....	2½–3 in.	5 c.c.	45 min.	Partial 24–38 hrs.	*ca.* 2 hrs.	Neg. in 38 hrs.
2.....	1–3 in.	....	1½ hrs.	Neg. in 34 hrs.	2 hrs.	Neg. in 34 hrs.
3.....	2¼ in. (*ca.*)	8 c.c.	30 min.	Neg. in 48 hrs.	2–3 hrs.	Sol. in 24 hrs.
4.....	2 in. (*ca.*)	6 c.c.	19 min.	Neg. in 2¼ hrs.	*ca.* 2 hrs.	Sol. in 2¼ hrs.
5.....	1–2½ in.	4 c.c.	2¼ hrs.	Neg. in 24 hrs.	*ca.* 2 hrs.	Neg. in 24 hrs.
6.....	1–3 in.	4 c.c.	2¼ hrs.	Neg. in 24 hrs.	*ca.* 2 hrs.	Neg. in 24 hrs.

The rapidity of the filtration process in trials Nos. 3 and 4 made it quite probable that some leakage of the sacs occurred. With the exception of these two, the results indicate that pepsin cannot pass through the collodion filter.

EXPERIMENT IV.

Dialysis through collodion sacs.—As with other enzymes heretofore stated it will be seen that although pepsin does not filter through the collodion wall, it is dialyzable through that membrane. The following experiment will illustrate the dialysis of pepsin:

Seven thin collodion sacs were prepared as before described and were labeled *S*, *C*, *C'*, 1, 2, 3, 4, and 5, respectively. Each sac was immersed in a test-tube on foot containing 20 c.c. of a 0.7 per cent. NaCl, acidulated with 0.4 c.c. of a 10 per cent. HCl, and 0.1 g. fibrin. Each of the sacs *C* and *C'* contained 5 c.c. of a 1:5,000 pepsin solution, previously boiled for five minutes, and served as controls. In each of sacs 1–5, inclusive, were placed 5 c.c. of a freshly prepared 1:5,000 pepsin solution. All the tubes were set aside in an incubator at 37°.

When observed at the end of twenty-eight hours, the solution of the fibrin was complete in Tube 4. A small sediment remained in Nos. 1, 2, and 5. A small lump of undissolved fibrin remained in No. 3. The fibrin placed in this tube consisted of a solid piece, and not of shreds as in the other tubes. The controls showed no solution. At end of forty-eight hours the solution of the fibrin in sacs 1–5, inclusive, was practically complete, whereas in the control tubes there remained a comparatively large sediment.

The sacs were tested at the close of the experiment, and were found free from leaks.

The foregoing details show (1) that pepsin is removed from dilute solution by filtration through hardened filtered paper; (2) that this removal is probably not due to fixation by the paper; (3) that aeration and surface action are injurious to pepsin; (4) that pepsin is removed from solution by the Berkefeld bougie and by the collodion filter; (5) and that it readily dialyzes through the collodion sac.

PANCREATIN.

The pancreatin used in these experiments was the commercial preparation of Parke, Davis & Co. The solutions employed, except when otherwise indicated, were of a strength of 1 : 500 in 0.7 per cent. NaCl. Before use they were filtered through a single thickness of ordinary filter paper.

The pancreatin was tested by adding to 5 c.c. of the solution 0.08 c.c. of a 10 per cent. Na_2CO_3, and 0.1 g. fibrin. One hundred and ninety cubic centimeters were filtered in each instance. Control tests were made with the unfiltered solution. Blank control consisted of 0.1 g. fibrin in 5 c.c. of a 0.7 per cent. NaCl containing 0.08 c.c. of a 10 per cent. Na_2CO_3.

EXPERIMENT I.

Filtration through paper.—The following table gives the results obtained on filtering the pancreatin solution through a series of hardened filter paper. The tests were made at a temperature of 36°. A common control served for the "6×2" and "8×2" filtrates. Blank controls, it may be added, were negative in every trial.

TABLE XXXV.

No.	Duration of Filtration	Time Required to Dissolve Fibrin by						
		"2×2"		"4×2"		"6×2"		"8×2"
		Control	Filtrate	Control	Filtrate	Control	Filtrate	Filtrate
1	3 hrs. 15 min.	8–9 hrs.	*ca.* 10 hrs	8–9 hrs.	10–18 hrs.	8–9 hrs.	31–42 hrs.	Neg., 18 hrs
2.....	2 hrs. 15 min.	7–10 hrs.	7–10 hrs.	*ca.* 10 hrs	10–21 hrs.	9 hrs.	22–23 hrs.	Neg., 48 hrs
3.....	2 hrs. 20 min.	7½–9 hrs.	*ca.* 9 hrs.	7–8 hrs.	8–21 hrs.	6–7¾ hrs.	21–24 hrs.	Sol. in 48–72 hrs.

It is evident from these tests that pancreatin is removed from its solution by hardened filter paper.

Subsequent aeration experiments failed to demonstrate the fixation of enzyme. Aeration was found to have a destructive effect on the enzyme, which destructive effect was increased by the surface action of paper and of garnets.

EXPERIMENT II.

Filtration through the Berkefeld filter.—The results obtained by Fermi and Pernossi and others, in connection with the filtration of pancreatin through porous filters have already been noted. Below are tabulated the results of a series of filtrations through the Berkefeld bougie. The filtrates and controls were tested in the manner described above. One hundred and ninety cubic centimeters of the 1:500 enzyme solution were filtered in each instance. The digestion temperature was 36–37°.

TABLE XXXVI.

No.	Pressure	Time of Filtration	Time Required to Produce Solution of Fibrin by		
			Control		Filtrate
			Blank	Pancreatin	
1.....	22¾ in.	40 sec.	Negative	5 hrs.	24–25 hrs.
2.....	28⅜ in.	55 sec.	Negative	7+hrs.	24–30 hrs.
3.....	28 in.	75 sec.	Negative	8 hrs.	¾ diss. in 48 hrs.
4.....	50 lbs.	16 sec.	Negative	9½–11½ hrs.	24 hrs.
5.....	50 lbs.	16 sec.	Negative	8¼–10¼ hrs.	35 hrs.
6.....	100 lbs.	About 12 sec.	Negative	8 hrs.	50 hrs.
7.....	100 lbs.	About 10 sec.	Negative	8 hrs.	30–45 hrs.
8.....	100 lbs.	About 10 sec.	Negative	8 hrs.	30–45 hrs.

It will be observed that, as in the case of pepsin, the bougie becomes clogged by successive filtration.

After the second filtration at 50 pounds' pressure, the bougie in use was accidentally broken. The subsequent filtrations at 100 pounds were then performed with a new bougie. During the process of filtration the tube connecting the filtering apparatus with the pressure tank broke, after 98 c.c. of the liquid had passed through. The succeeding portion of liquid which filtered through at gravity pressure was collected separately. The time of filtration at 50 pounds' pressure was seven or eight seconds. The gravity filtration which followed gave 3–4 c.c. per minute. The filtrates and controls were tested as before with the following results:

Blank control — negative in 48 hrs.
Pancreatin control — solution in 10–21 hrs.
Gravity filtrate — negative in 48 hours.
50 pounds' filtrate — negative in 48 hrs.

Evidences of bacterial growth in these tubes appeared at the end of forty-eight hours, and hence the observations were discontinued. There was in this instance no passage of pancreatin through the bougie. This same bougie was used in 100-pound filtrations tabulated above.

EXPERIMENT III.

Filtration through collodion sacs.—The collodion filter is impermeable to trypsin as the following table will show.

TABLE XXXVII.

No.	Pressure	Time of Filtration	Amount Filtered	Temp. of Digestion	Time Required to Dissolve Fibrin by		
					Control		Filtrate
					Blank	Pancreatin	
1.....	1½–3 in.	2 hrs.	5 c.c.	36°	Neg.	6½–8 hrs.	Neg. in 48 hrs.
2.....	2–3⅛ in.	2 hrs.	5½ c.c.	37°	Neg.	7+hrs.	Neg. in 72 hrs.
3.....	2–2½ in.	1½ hrs.	5½ c.c.	37–38°	Neg.	*ca.* 8 hrs.	Neg. in 72 hrs.

Dialysis through collodion sacs.—Unlike the enzymes already studied, pancreatin does not dialyze through the collodion sac. The dialysis experiments were performed exactly as in the case of preceding enzymes. The tubes were kept under observation in one instance as long as eleven days, but at no time was there any evidence of dialysis.

Inasmuch as the commercial pancreatin was employed for the preceding experiments, it was deemed desirable to check these with a freshly prepared trypsin solution. Accordingly, a fresh beef pancreas was ground up in a sausage machine, and the pulp was extracted with water. The filtered solution was precipitated with alcohol, and the precipitate was then digested with glycerin. The glycerin extract thus obtained was found to possess a marked proteolytic action. All attempts to dialyze this trypsin solution or to filter through the collodion sac gave negative results, as did the commercial preparation.

These experiments show (1) that pancreatin is removed from its solution by filtration through hardened filter paper; (2) that this removal evidently is not due to fixation; (3) that the enzyme is injuriously affected by aeration and by surface action; (4) that it passes in varying degree through the Berkefeld bougie, in one instance being totally retained; (5) that it does not filter through the collodion sac; (6) and that, unlike all the other enzymes experimented upon, it does not dialyze through the collodion sac.

CONCLUSIONS.

The results obtained in this study may be advantageously and briefly summarized as follows:

I. The removal from solution by paper filtration of ptyalin, rennet, pepsin, and pancreatin.

II. The fixation of ptyalin by hardened filter paper.

III. The injurious effect of aeration and of surface action on taka diastase, rennet, pepsin, and pancreatin.

IV. The passage of ptyalin and taka diastase through the Berkefeld bougie; the absolute retention of rennet, even under high pressure, by the Berkefeld bougie; the partial retention of pepsin; and the partial retention (in one case total retention) of pancreatin.

V. The total retention of rennet by the Chamberland bougie.

VI. The total retention of ptyalin, rennet, pepsin, and pancreatin by the collodion filter, and the partial passage through this filter of taka diastase.

VII. The dialysis of ptyalin, taka diastase, rennet, and pepsin, but not of pancreatin, through the collodion membrane.

I wish to express my obligation to Professor Novy, at whose instance this work was undertaken, and whose advice and suggestions have been of valuable assistance throughout the work. Acknowledgments are also due to the Rockefeller Institute for Medical Research for the support necessary to carry on this investigation.

REFERENCES.

1. MUSCULUS, *Compt. rend. Ac. Sc.*, 1874, 78, p. 132; 1876, 82, p. 333.
2. LEA, *Jour. of Physiol.*, 6, p. 136.
3. MIQUEL, *Fermentation ammoniacale et ferments de l'urée* (Paris: Carré et Naud, 1898), p. 262.
4. WORTMANN, *Bot. Zeitung*, 1890, 48, pp. 581, 597, 617, 633, 657.
5. BROWN AND MORRIS, *Jour. Chem. Soc.*, 1893, 63, p. 634.
6. BROWN AND HERRON, *Proc. Roy. Soc.*, 1880, 30, p. 399.
7. VINES, *Ann. of Bot.*, 1891, 5, p. 410.
8. KASTLE AND LOEVENHART, *Amer. Chem. Jour.*, 1900, 24, p. 498.
9. SZUMOWSKI, *Arch. de Physiol.*, 5e Série, 1898, 10, p. 160.
10. EFFRONT, *Les enzymes et leur applications* (Paris: Carré et Naud, 1899), p. 21.
11. CHEVREUL, *Compt. rend. Ac. Sc.*, 1853, 36, p. 986.
12. GORSLINE, *Contributions to Medical Research* (Dedicated to Dr. V. C. Vaughan: Ann Arbor, Mich.: Wahr, 1903), p. 390.
13. RODET AND GUÉCHOFF, *Compt. rend. Soc. Biol.*, 1900, 52, pp. 965–67.
14. CRENDIROUPOULIS AND RUFFER, *ibid.*, pp. 1109, 1110.
15. NOVY, *Laboratory Work in Bacteriology* (Ann Arbor: Wahr, 1899), p. 499.
16. GAUTIER, *Compt. rend. Ac. Sc.*, 1882, 94, p. 652.
17. FERMI AND PERNOSSI, *Ztschr. f. Hyg.*, 1894, 18, p. 106.
18. ACHALME, *Ann. de l'Inst. Past.*, 1901, 15, p. 739.
19. FERMI. See Fermi and Pernossi.
20. BUCHNER, *Die Zymasegärung* (Edward Buchner, Hans Buchner, and Martin Hahn; München and Berlin: R. Oldenbourg, 1903).
21. FERNBACH, *Ann. de l'Inst. Past.*, 1890, 4, p. 641.
22. EFFRONT, *Enzymes and Their Applications* (Translated by S. C. Prescott; New York: Wiley & Sons, 1902), p. 57.
23. EHRLICH AND MORGENROTH, *Berl. klin. Wchnschr.*, 1900, 37, p. 682.

24. VEDDER, *Jour. Med. Research*, 1903, 9, pp. 475–501.
25. OPPENHEIMER, *Die Fermente und ihre Wirkungen* (Leipzig: Vogel, 1900), p. 37.
26. LEA, *Proc. Roy. Soc.*, 1883, 36, p. 55.
27. LEA AND DICKINSON, *Jour. of Physiol.*, 1890, 11, p. 310.
28. ROBERTS, *On the Digestive Ferments*, etc. (London: Smith, Elder & Co., 1880), p. 67.
29. CONN, *Centralbl. f. Bakt.*, 1892, 12, p. 223.
30. KORSCHUN, *Ztschr. f. Physiol. Chem.*, 1903, 37, p. 366.
31. NOVY, *Loc. cit*, p. 471.
32. DUCLAUX, *Traité de microbiologie* (Paris: Masson et Cie, 1900), 2, p. 271.

FATAL INFECTION BY A HITHERTO UNDESCRIBED CHROMOGENIC BACTERIUM, BACILLUS AUREUS FOETIDUS.*

MAXIMILIAN HERZOG,
Manila, P. I.

(*From the Government Biological Laboratory, Manila, P. I.*)

THE fauna and flora of the tropics being in general different from that of the more temperate latitudes, in genera as well as in species, we may reasonably expect that this observation will hold good also with reference to the very lowest forms of life—bacteria and protozoa—without, however, being unmindful of the fact that certain families and even species are distributed over an enormous territory under the most varied conditions of life.

We can hardly expect to find that those bacteria and protozoa which are strict parasites of widely distributed races are limited in area, unless it be that they depend in certain stages of their life-cycle upon an intermediate host, itself confined to certain areas. To cite an example: The tubercle bacillus is found practically wherever an easily susceptible host of this parasite—like the human being—dwells. Hemameba malariae—likewise a parasite of man—is not so widely distributed, because it depends for its dissemination upon an intermediate host—the anopheles, the distribution of which is not identical with that of man.

The *a priori* deduction that we shall find in the tropics certain bacteria and protozoa peculiar to this zone is therefore limited to those bacteria and protozoa which are either strictly saprophytic or parasitic in certain hosts, confined to the tropics themselves, or which only occasionally and under particularly favorable circumstances lead a parasitic life.

The observation to be recorded in this paper refers to a case of fatal human infection by a hitherto undescribed bacterium, which is evidently not very pathogenic in ordinary conditions and as a rule is probably a harmless saprophyte, which, however, in

* Received for publication March 23, 1904.

circumstances especially favorable, as in this instance, may become parasitic and cause a fatal issue. Examples of this type are of course not unknown in clinical medicine. The colon bacillus, usually a harmless saprophyte, living in the intestinal contents, occasionally invades the fluids and tissues of the human body and leads to acute or chronic fatal infections.

The case to be reported is as follows:

On February 19, 1904, the body of D. L., a Filipino, 40 years of age, was sent to the morgue at San Lazaro. All that was known about the case was that the person had died rather suddenly, and it was suspected that he had died of plague, having an open wound on his right leg, and a marked swelling of the inguinal glands of the right side. The postmortem examination was made eight hours after death. The findings were in short as follows:

The body of a well-developed man, about 40 to 45 years of age. Postmortem rigidity well marked; lividity well marked on dependent parts of the body, and extending somewhat toward the sides of the neck and throat. There is a swelling of the size of a hazelnut, firm and elastic, in the right inguinal region. There is no fluctuation and no edema in the neighborhood. The skin is intact over this area and of normal color. In the right popliteal space there is an ulcerated surface $5 \times 1\frac{1}{2}$ cm. in size, the long diameter being in the long axis of the limb. The ulceration is several millimeters deep, has sharp, somewhat raised edges, and the granular surface is covered with a small amount of sero-fibrinous exudate; iodoform has been dusted freely on the wound. The condition of the ulcer suggests that the surface has recently been curetted and its edges trimmed.

The serous membranes are shining and transparent. There is a very small amount of serous fluid in the thoracic and abdominal cavities. Hemorrhagic spots are not found anywhere. The pericardium, and the heart and large vessels appear normal. The lungs are slightly hyperemic, otherwise normal: bronchi, trachea, and larynx normal; epiglottis slightly injected.

Spleen normal in size, consistency, and color; trabeculae a little more marked than usual.

The kidneys show a marked injection and are of a deep pinkish-purple hue. The capsule is smooth, even, and transparent, and peels off easily. The surface appears slightly granular. The glomeruli are strongly injected and surrounded by a much paler, somewhat grayish-white tissue. The pyramids are likewise of a deep, pinkish-purple color. The relation between cortex and medulla is normal. The mucous membrane of the pelves is smooth and shining. Suprarenals normal.

The liver is normal in size and rather firm in consistency. Capsule is smooth and transparent, except that on the upper surface are a few small, irregular, dull, slightly raised perihepatitic areas. On section the color is brownish, the centers of the lobules being rather grayish-white.

The gall bladder is normal and contains a moderate amount of turbid, greenish-yellow bile; no stones. The pancreas is perhaps somewhat firmer in consistency than usual, otherwise normal.

Stomach and small intestines: mucosa slightly congested, otherwise normal. Large intestine and appendix are normal.

Three of the inguinal glands of the right side are enlarged to twice their normal size in all their diameters. On the left side they are slightly enlarged. The glands of the right side are quite firm in consistency, not injected, and rather pale. The cervical glands are slightly enlarged and moderately congested.

Smears from the organs show a small number of what appears to be a small diplococcus or diplobacillus. There are no organisms found showing the characteristic morphology and staining properties of the plague bacillus.

It was therefore decided—a decision having to be made shortly after the postmortem examination in order to determine the course to be taken as to the body and the house from which it came—that the case was not one of plague infection.

The anatomical diagnosis reads as follows:

Passive congestion of the kidneys and liver. Acute interstitial hepatitis. Enlargement of the inguinal and cervical glands.

Cause of death: Remote, iodoform poisoning (?); immediate, terminal diplococcus infection.

It may be stated that inquiries as to the history of the case and as to the possibility of iodoform poisoning were made. As usual in the case of the lower class of Filipinos, only a very fragmentary, unsatisfactory history could be obtained. Dr. Christensen, health inspector of the district from which the body was sent for postmortem examination and diagnosis, had never seen the deceased alive, but learned that he had not been perfectly well for about four months, that he had had an open wound on his leg, and that iodoform had been used freely on it for about eight days. No history of any symptoms of iodoform poisoning could be obtained. The liver was examined for iodin in the chemical laboratory, but no iodin could be found.

DESCRIPTION OF THE BACILLUS ISOLATED.

During the postmortem examination glycerin agar tubes were inoculated with the usual precautions.

Two tubes inoculated from the liver developed pure cultures of a short, small bacillus, which produced a golden yellow pigment.

One tube from the heart's blood developed the same organism, but it was, as shown on the third day, contaminated.

One tube inoculated from the spleen remained permanently sterile. None of the tubes developed the plague bacillus.

Morphology.—Short bacilli with rounded ends, varying much in size. They are from 0.6 μ to 2 μ long; on an average 1.4 μ long. The larger individuals of 2 μ are rather scarce. In thickness the bacilli vary from 0.55 μ to 0.8 μ. Proportion of length to thickness usually 2 : 1. The organism presents itself frequently as a diplobacillus. A large number of them are short, making them look much like diplococci. Occasionally there are found small individuals which are not materially larger than 0.5 μ and almost spherical, so that it is hard to distinguish them from true diplococci. The bacilli possess a capsule of moderate size, which can be demonstrated by Muir's method. They do not form large chains, even groups of four in a chain being but rarely seen. Spore formation is not observed. The organism is not motile.

Staining properties.—The bacilli are readily stained by the watery aniline stains and easily overstained by the more intense solutions (carbol-fuchsin, carbol-thionin). When lightly stained, not all of the bacilli take the stain uniformly; but in some cases the stain acts in such a manner that an unstained space remains in the center. It is not demonstrable that this polar staining is due to the presence of Ernst-Babes polar granules, because Neisser's methylene blue Bismark brown stain does not show any such granules satisfactorily, though there appears to be a slight tendency at the poles of the bacillus to take up some of the blue stain. The bacillus resembles somewhat the short type of the pseudodiphtheria bacillus. A certain similarity also exists between this bacillus and that of plague, though the similarity is not great. Gram's method decolorizes the bacillus.

Cultural peculiarities.—The organism produces on all solid media tried an intense golden-yellow pigment, which is practically identical in color with the pigment formed by Staphylococcus pyogenes aureus. Distinct colonies in 20 per cent. gelatin plates are quite difficult to obtain, because the organism liquefies the gelatin with great rapidity. Twenty per cent. gelatin stab cultures after 24 hours are fluidified largely. The liquefaction comprises the entire extent of the upper strata. There is some growth along the line of the stab, but not much. The liquefied gelatin looks very cloudy and after 24 hours a dense scum is formed on its surface. Individual colonies are best studied on agar plates. On agar and glycerin agar the organism after 24 hours forms a moist, raised, golden-yellow growth. The individual colonies are more or less round and likewise moist and raised, with a somewhat undulating surface. The margins are smooth. In spreading, the colonies become confluent. The development on glucose agar is identical with that on ordinary agar. No gas formation occurs. On three per cent.

salt agar the growth is possibly a little slower, although not very much so. The bacilli raised on this medium stain as usual, and do not show the involution forms so characteristic of the plague bacillus. On lactose agar the growth is similar to that on the other agars. There is no gas formation. The development on the surface is more rapid than in the depth of the stab. In lactose litmus agar the color begins to turn after 24 hours and is quite distinctly red after 48 hours. Broth after 24 hours is strongly clouded, and after 48 hours a pellicle has been formed on the surface. On potatoes a typical luxuriant growth is observed after 24 hours. Litmus milk is slightly reddened after 24 hours, and is strongly so after 48 hours. Coagulation takes place only at the end of several days. The organisms develop typically under anaërobic conditions in both a nitrogen and a hydrogen atmosphere. All cultures whether aërobic or anaërobic have a fetid, cheesy, and somewhat cadaverous smell.

The thermal death-point of the organism was determined to be 62° C. An exposure of ten minutes at this temperature destroyed all the bacilli, while 61° C. acting for ten minutes left a number alive.

The name "Bacillus aureus foetidus" selected for this micro-organism emphasizes two of its prominent characters—its chromogenic and malodorous properties.

PATHOLOGICAL HISTOLOGY.

Pieces of tissues were placed in Zenker's solution, imbedded in paraffin and stained with hematoxylin eosin, eosin alkaline methylene blue, and by Gram's method.

Liver.—The boundaries of the lobules are well marked, since the interlobular veins are surrounded by an inflammatory cellular infiltration. In quite a few places this inflammatory process must have been going on for some time, since here the interlobular tissues show a number of fusiform connective tissue cells and fibers. When examined with the immersion lens, it is seen that the cellular exudate consists mainly of small round cells of the lymphoid type: here and there a plasma cell is seen. Quite a number of the small round cells show karyokinetic figures, demonstrating that a lively proliferation has been going on in the inflammatory foci. The latter contain a considerable number of small bacilli found in irregular groups, in groups of two, and in small chains. This micro-organism does not generally stain very well, even in methylene blue. Some, however, keep the dye fairly well.

The liver cells show a marked, though not advanced, degree of fatty change. This process is perhaps most marked in the center of the lobule; though it may be quite diffusely distributed in places. The liver capillaries are congested.

Kidneys.—The majority of the glomeruli appear normal; some, however, show an increase in the nuclei of the endothelial lining of the glomerular capillaries, while in others there is a more or less marked thickening of the capsules of Bowman. We also see beginning fibrosis in the interior of the tufts, and there may be a complete obliteration of the capillaries. In the

neighborhood of such glomeruli as show more or less advanced changes and between the convoluted tubules, are inflammatory foci. *These foci consist mostly of small round cells of the lymphoid type.* There are present, however, some plasma cells and plasma "mast cells," and a considerable number of eosinophilic polynuclears. These foci likewise show the small, generally poorly stained bacilli. The epithelial cells lining the uriniferous tubules show cloudy swelling or vacuolation, with loss of the nucleus. Most tubules contain a granular material, some contain hyaline casts. The renal blood vessels are all densely filled with blood.

Neither the liver nor the kidneys show any areas of marked coagulation necrosis; nor are areas of blood extravasation encountered.

Lymph glands.—The inguinal lymph nodes show a marked increase in fibrous connective tissue in the capsule, in the trabeculae, and around the individual blood vessels. The follicles themselves, however, show no marked fibroid changes, and the differentiation between the peripheral zone and the central proliferating center is well preserved. Karyokinetic figures are seen here and there in the proliferating center. Occasionally an eosinophilic polynuclear is encountered. Plasma cells and plasma "mast cells" are also seen. Bacilli like those found in the liver and kidneys are present, forming little groups here and there among the cells. The blood-vessels of the lymph nodes are generally well filled.

No changes are found in the lungs. The pancreas is normal except for slight increase in the interlobular connective tissue.

The myocardium shows some fragmentation, brown atrophy, and fatty changes.

ANIMAL EXPERIMENTS.

February 26, 1904, a small monkey (Macacus philippiniensis) was given an intraperitoneal injection of two to three c.c. of a 24-hour broth culture of the bacillus. There was only a slight reaction, and the animal was well at the end of one month.

March 1, 1904, a good-sized, full grown rabbit was given an intraperitoneal injection of two to three c.c. of an emulsion of a two-days' agar culture in sterile water. The result negative.

A negative result was obtained in a half-grown wild gray rat inoculated subcutaneously with a platinum-loop of a fresh agar growth.

It appears from these animal experiments that Bacillus aureus foetidus is not highly pathogenic.

CONCLUSIONS.

Beyond doubt, Bacillus aureus foetidus, the bacterium now described, was the cause of death in the case here reported. Experiments show that the bacillus is not a highly pathogenic microorganism, because single inoculations of moderate doses brought about only slight reaction in the animals experimented

upon. Perhaps inoculations repeated during a longer period might bring about more serious result.

It is probable that Bacillus aureus foetidus is ordinarily a saprophyte. In the case reported it may simply have lived for some time in the necrotic tissues of a neglected ulcer, and may have become modified in this environment until it finally gained entrance into the juices and tissues of the patient. From the lymphatic system it entered the blood current, reached the liver and kidneys, and led to subacute and somewhat chronic interstitial fibroid processes and parenchymatous degenerations. To the anatomical diagnosis made at the postmortem table must be added, as shown by microscopic examination: Beginning interstitial and marked parenchymatous nephritis, as well as beginning, though slight, brown atrophy and fatty degeneration of the myocardium.

OBSERVATIONS ON THE BIONOMICS OF ANOPHELES.*

E. O. Jordan and Mary Hefferan.

The striking recedence of malaria in certain portions of the world and its equally noticeable extension in others are still in part unexplained phenomena, and offer a field for investigation that involves a consideration of many strictly biological problems. Viewed in its broader aspects, the successful conduct of a campaign against malaria rests upon a proper comprehension of the relation of three organisms belonging to widely separated groups of the animal kingdom. The importance of understanding the biological or ecological features of the problem can hardly be overestimated. The possible existence of natural causes favoring or hindering malaria is a matter upon which studies in the field may be able to shed light. An instance of the fruitful outcome of general observations is found in Theobald Smith's[1] suggestive remarks on the natural history and probable mode of spread of tertian malaria in Massachusetts. With these points in mind the writers have undertaken a series of detailed observations on the bionomics of the malaria-bearing mosquito.

The following observations on Anopheles have been carried out partly in the vicinity of Chicago, partly in a small country town in western Michigan (Eastmanville, about twenty miles west of Grand Rapids), and partly in a locality in New Hampshire (Shelburne) already described by one of the writers.[2]

Anopheles larvæ were collected at thirty-four different points in and about Chicago, including thirteen stations within the city limits. Breeding pools were found in all quarters of the city, and in the suburbs on the north, west, and south sides. The adult mosquitoes sometimes invade rather thickly settled parts of the city, and have been captured in houses within two blocks of

* Received for publication Dec. 1, 1904. Aided by a grant from the Elizabeth Thompson Science Fund.

1 *Bost. Med. and Surg. Jour.*, 1903, 149, p. 57.

2 *Jour. Med. Research*, 1902, 7, p. 1.

the University of Chicago campus. The dominant species raised from larvæ taken in this vicinity has been A. punctipennis. In 1902, 59 individuals belonging to this species were bred, 29 of which were males and 30 females, while only 19 individuals of A. maculipennis developed (5 males, 14 females). In 1903 the number of the latter species was much smaller (A. punctipennis, 32 males, 50 females; A. maculipennis, 1 male, no female). A. punctipennis seems to be the more common species in eastern Massachusetts,[1] as is also the case in at least one region in northern New England.[2] A record kept of the breeding places about Chicago, and the species developing from the larvæ captured showed that in four different bodies of water the larvæ of the two species were found together. Those reared from the larvæ and pupæ captured were divided between the two species as follows:

TABLE I.

Locality Number	A. punctipennis	A. maculipennis
5	4	1
9	15	5
14	1	1
17	1	8

Larvæ collected from seven different stations yielded A. punctipennis alone, while those collected from four stations produced only A. maculipennis. Our observations afford no reason for believing that the larvæ of A. maculipennis are reared with any more difficulty under artificial conditions than those of A. punctipennis. In fact it appears from our experience that larvæ that are 6–7 mm. in length when captured almost always pupate and develop normally when afforded ordinary care and attention. The figures given above are based entirely upon the results of raising these larger larvæ and pupæ. As a matter of fact, in western Michigan, where A. maculipennis was the more abundant form, no difficulty was experienced in rearing the larvæ. The

[1] *Bost. Med. and Surg. Jour.*, 1903, 149, p. 88.

[2] *Jour. Med. Research*, 1902, 7, p. 1; cf. also DYAR, *Proc. Entomol. Soc. Wash.*, 1903, 5, No. 2.

disparity in the numbers of the two species bred from larvæ and pupæ must therefore be regarded as indicating the relative scarcity of A. maculipennis in the vicinity of Chicago, and in the neighborhood of Shelburne, N. H.

In the third locality studied (western Michigan), A. maculipennis was by far the more common species, as shown in the following tables:

TABLE II.

	Imagoes Captured			
	A. maculipennis Males	A. maculipennis Females	A. punctipennis Males	A. punctipennis Females
1902	75	122	2	9
1903	42	156	2	5
1904	295	436	8	11

TABLE III.

	Bred from Larvæ and Pupæ			
	A. maculipennis Males	A. maculipennis Females	A. punctipennis Males	A. punctipennis Females
1903	33	32	1	4
1904	22	21	13	15

The distribution of the two species is a matter of some interest, since it has been proved that there is probably a difference in their ability to transmit malaria. A. maculipennis is the dominant species in Italy, Greece, Spain, and parts of France, and according to Laveran,[1] plays throughout Europe the most important rôle in the propagation of malaria. A. punctipennis is confined to North America. An attempt made by Berkeley and Smith[2] to infect A. punctipennis led to no definite outcome; Hirschberg[3] has since conducted more elaborate experiments upon the two common species of Anopheles by feeding them with the blood of patients known to contain the parasite of aestivo-autumnal malaria. The following results were obtained:

[1] *Comp. Rend. de la Soc. de Biol.*, 1904, March 4, p. 325.

[2] *New Jersey Agricultural College Expt. Station Rept.*, 1902, p. 512.

[3] *Bull. Johns Hopkins Hosp.*, 1904, 15, p. 53.

TABLE IV.

	Number Fed	Number Infected
A. maculipennis....	48	8
A. punctipennis.....	58	0

A similar difference in susceptibility to infection has been shown to exist between two common Anophelines in India, M. rossi and M. culicifacies (Christophers and Stephens).

Many localities in the United States show a marked disparity in the proportion of A. maculipennis and A. punctipennis. Bashore[1] found a preponderance of A. maculipennis (133 to 7) in a suburb of Harrisburg, Pa. Mention has already been made of the difference in the relative abundance of the two species in the localities observed by the writers, a difference furthermore that persisted through three seasons. A similar difference has been noted in the neighborhood of Boston (T. Smith) and Baltimore (Hirschberg). Hirschberg has pointed out that the distribution of the two species in the vicinity of Baltimore as previously determined by himself and Dohme[2] coincides in a general way with the occurrence of malaria-infected districts. The disease prevails where A. maculipennis has its breeding places, but is absent from districts where A. punctipennis abounds.

In but one of the three localities (Michigan) examined by us is A. maculipennis present in notable numbers. It may be remarked that this is also the only one of the three in which there is any history of marked malarial prevalence. The character of the region where A. maculipennis is found so abundantly is therefore of interest.

A. *The Locality.*—The observations were made in and about the village of Eastmanville, in western Michigan, during the summers of 1902, 1903, and 1904. A white settlement has existed at this point, upon the north bank of the Grand River, twenty miles (river measurement) from its debouchement into Lake Michigan, since about 1840. Prior to that date, and for some time afterward, Indians of the Ottawa and Pottawatomie tribes had villages near this part of the river. The population of the village, never more than a few hundreds, is at present about 150.

[1] *Med. Record*, 1901, 59, p. 173.

[2] *Bull. Johns Hopkins Hosp.*, 1902, 13, p. 45.

The soil of the district is clay, covered by a few feet of sand for the most part, but occasionally cropping out. The country-side is almost entirely under cultivation or in pasture land; little or nothing remains of the marshes or of the heavy timber which formerly covered the area. The north bank of the river, upon which the village lies, rises with somewhat more marked declivity than the south, which is sandy, the channel being here, for two miles, on the north shore. The stream is at this point thirty rods wide; the vegetation of the shores is characterized by low willows and wild rice. North from the river run several ravines, dry in summer except for occasional spring-fed pools. Opposite the village, and separated from the river by a strip fifteen rods wide, part sand and part marshland, is a deep currentless bayou, mud-bottomed, spring fed, ten rods wide and two miles long. With the exception of a few rods, its shores are timbered; the vegetation is that of a spring-fed lake.

B. *Malarial History.*—This part of Michigan was in earlier times, according to tradition, one of the worst malarial districts in a highly malarial state. No records except the general State Reports for western Michigan are available to show the former prevalence of malaria in Eastmanville, but the testimony of all the older settlers is unanimous. Everyone in the district was a sufferer, and everyone who came to the district expected to contract the disease. From the strongest of the laboring men to the infant born on the mother's ague day, the entire population was subject to chills and fever.

Such conditions do not now exist. The Michigan State Board of Health Reports contain interesting statistics regarding the decrease of malaria in Michigan during twenty-three years.

TABLE V.

Weekly Reports of Diseases in Michigan.

per cent. of reports which showed intermittent fever.

1877....75%	1884....65%	1891....36%	1898....19%
1878....82	1885....59	1892....27	1899....17
1879....82	1886....54	1893....24	1900....16
1880....82	1887....48	1894....24	1901....14
1881....82	1888....45	1895....22	
1882....71	1889....43	1896....19	
1883....69	1890....41	1897....17	

In 1879, 1880, and 1881, intermittent fever was the most prevalent of all diseases in Michigan; in 1881 the western division of the State, including the locality described above, showed a greater prevalence of malaria than other parts of the State, 90 per cent. of all weekly reports recording the disease as under the observation of the physician making the report. But according to the statements of local physicians the disease has been very infrequent in and about Eastmanville for the last few years. One physician had observed during six years but three cases presenting a typical malarial history; one of these occurred in the summer of 1889, one in 1902, and one in 1904. All three patients lived on the bank of the river, at distances of several miles from one another.

The question of course arises whether this decrease in malaria has been paralleled by a decrease in the number of the malaria-carrying mosquitoes.

In the absence of data, no definite statement can be made regarding the number and virulence of mosquitoes in the earlier years of the settlement, except the very general one that they were much "more numerous" than at present, and that they were very large. They were felt as so real a pest that the farmers would often leave the unscreened houses to sleep in the barns and haylofts, which were not frequented by the mosquitoes.

THE BREEDING-PLACES.

Stephens[1] has called attention to the practical importance of noting any difference in the breeding-grounds of the various species. In India M. culicifacies (known to convey malaria) "loves clear, fresh water of streams, rivers, canals, and other moving waters where it occurs in myriads," whereas M. rossi (probably not a malaria carrier) breeds only in shallow pools or puddles some inches deep. This difference in breeding habits naturally assumes high importance in any attempt to lessen malaria through destruction of breeding-places.

If there is a similar difference in the breeding-places of A. maculipennis and A. punctipennis, the task of restricting the spread of malaria in some regions may be materially simplified. In the present instance it was found that the favorite breeding-places of A. maculipennis and A. punctipennis, although close together, were of quite different character, as shown by the following facts relating to the distribution in Western Michigan.

Dipping for mosquito larvæ was carried out in the springs and spring-fed pools of the northern ravines, along the river shore, in the bayou, and in rain-water barrels of the village. In all of these places, with the exception of the bayou, Anopheles larvæ were found in abundance at some time during three consecutive summers. The distribution was more extensive in 1902, merely because the early part of the summer was wet, and water stood longer in the springs and ravines. In 1903 the early part of the summer was dry, so that by the middle of August two springs and the river shore only were left as breeding-places in the immediate vicinity of the village. In the river the larvæ of A. maculipennis are found regularly at certain places along the north shore, where abundant food and quiet are ensured by the wreckage and masses of river weed which lie a few inches below the surface of the water. Here, with the river running almost due west, the larvæ are exposed to direct sunlight. They were not found along the more shaded south shore. A curious instance of choice of breeding-places occurred in the summer of 1904. A small stream running to the river had, during the spring, a course of a mile or so down the ravine, but by August 1 it had dried to a few pools of the following

[1] *Brit. Med. Jour.*, Sept. 17, 1904, p. 629.

character: (*a*) River inlet, 40 feet long and 7 feet wide, shaded by willows, bottom sand and mud, no larvæ; (*b*) Ten feet above this inlet a pool 40 feet by 4 feet in area, some 15 inches deep, entirely without shade or vegetation, no larvæ; (*c*) Only three feet from this second pool another pool of clear water, 5 feet by 3 feet in size, and 6 inches deep, shaded in the morning. Duckweed abounds here, and a quantity of green algæ cover the sticks and stones of the bottom: the pool swarms with Culex and Anopheles punctipennis larvæ; (*d*) Thirty-five feet above (*c*) is an "iron" pool with a deposit of iron; it is about 9 feet by 9 feet, 10 inches deep, partly shaded by bushes and a huge log. It contains no vegetation and no larvæ.[1] Of these four pools the smallest one, in the center of the row, is the only one containing Anopheles larvæ. *These larvæ are exclusively those of A. punctipennis, while in the river only a few feet from here, A. maculipennis is abundant.*

Another noticeable feature of the distribution is the entire absence of all mosquito larvæ from the bayou, although the water is currentless, the vegetation abundant, and the light and shade favorable. It is probable that the duckweed and lily pads cover the surface too closely in the shallow water near the shore. As pool (*c*) above described became choked with duckweed later in the season, the larvæ disappeared.

The following table shows the undoubted selection of different breeding-places by A. maculipennis and A. punctipennis:

TABLE VI.

ANOPHELES BRED FROM LARVÆ AND PUPÆ.

	A. MACULIPENNIS		A. PUNCTIPENNIS	
	Males	Females	Males	Females
Collected from spring-fed pools	0	0	30	35
Collected from river	46	47	1	4

The fact that the breeding-places are only a few feet apart renders especially remarkable the specific preference displayed. The predominance of A. punctipennis in the other two regions that were examined is in accord with the observations in Michigan. A. punctipennis shows a predilection for spring-fed pools, and, in localities where these abound, is the chief species. Hirschberg and Dohme[2] observed a rather definite geographical distribution of the two species in the vicinity of Baltimore, A.

[1] C. B. DAVENPORT has already mentioned the absence of larvæ in "iron" springs and in completely shaded pools, *Reports on Plans for the Extermination of Mosquitoes on the North Shore of Long Island, North-Shore Improvement Assoc.*, 1902, p. 40.

[2] *Loc. cit.*

maculipennis being found on low ground in or near large bodies of water, while A. punctipennis was found in small, clear streams or springs on higher ground.

DIFFERENTIATION OF LARVAL FORMS.

We have attempted to discover some morphological character that would serve to distinguish the larvæ of A. punctipennis from those of A. maculipennis but without obtaining any satisfactory criterion. The larval markings vary materially in different localities and at different seasons. In New Hampshire one of us was able to distinguish the two species by the markings on the larval head, but the larvæ collected by the same observer in the vicinity of Chicago permitted no such differentiation, the head markings being variable and non-characteristic. This seems also to be the case in Eastern Massachusetts and in New Jersey.[1] On the other hand Dyar,[2] who found in studying the mosquitoes on Long Island, New York, that no larval distinction between A. punctipennis and A. maculipennis was discoverable, did observe a well-marked difference between the larvæ of these species the next summer at Center Harbor, N. H.

> Curiously enough all the larvæ (of A. maculipennis) found were alike and of a peculiar striking coloration. They were at once separable from A. punctipennis by being black with a straight, narrow white dorsal line, furcate on thorax. The white spotted A. punctipennis were never marked with such a uniform line.

From these facts it would seem that larval markings are swayed to a large extent by local environmental causes, and cannot be uniformly depended upon for species differentiation.

DURATION OF LARVAL LIFE.

A number of observers have experienced difficulty in raising young larvæ to maturity.[3] It is undoubtedly true that unless considerable care be taken in regard to food, sunlight, and character of the water, the mortality among young larvæ (1.5–4 mm.) will be high. We have found, for instance, that

[1] *New Jersey Agri. College Expt. Sta. Rep.*, 1902, p. 571.

[2] *Proc. Entomol. Soc. Wash.*, 1902, 5, No. 1.

[3] Cf. JOHNSON, *New Jersey Agri. College Expt. Sta. Rep.*, 1902, p. 373.

water drawn from a spring through zinc-lined pipes has a decidedly toxic influence upon the larvæ and causes the death of many in a relatively short time. Water taken directly from the same spring has no such injurious effect. Ordinary city tap water is also an unfavorable medium. On the other hand larvæ may be reared easily by keeping them in porcelain dishes holding several liters of water, and by allowing the direct sunlight to strike them, though only for a small part of the day. If water dipped immediately from the pond in which the larvæ were captured is used to replenish the aquarium, the food supply will usually be adequate. In case food in large quantities is desired, we have found pleurococcus scraped from moist boards a convenient source of supply.

Under the suitable conditions just outlined, eggs laid in the dishes hatched with great regularity in three days at the ordinary summer temperature. The rapidity of growth of the larvæ developing from the eggs of a single laying is very uneven. Sometimes nearly a month may elapse between the first and the last pupation of one and the same brood. Our observations showed a larval life of thirty-six to sixty-one days for A. maculipennis and twenty-five to forty-eight days for A. punctipennis. These are somewhat longer periods than those noted by Howard,[1] Berkeley,[2] Nuttall and Shipley,[3] and others, but the observations of these writers seem to have been made at considerably higher temperatures (Berkeley, 34–36° C.) than those that prevailed during the conduct of our observations. Owing to the fact that the porcelain dishes used in our experiments were kept in the open, the temperature represented more nearly such conditions as exist in the small pools that form the natural breeding-places. The chilling of the water at night in these pools is greater than in housed aquaria. The duration of larval life appears, therefore, to be chiefly a function of temperature, and may vary under ordinary circumstances anywhere from fifteen days to two months. Nuttall and Shipley state that larvæ caught in the middle of

[1] *Mosquitoes*, New York, 1901.

[2] *Laboratory Work with Mosquitoes*, New York, 1902.

[3] *Jour. of Hyg.*, 1901, 1902, 1903, 1, 2, 3.

August did not attain their full growth until November. It is yet a question whether the larvæ of A. maculipennis can hibernate. Those of A. bifurcatus can undoubtedly do so.[1]

PROPORTION OF SEXES.

As has been noted by all observers, the number of females captured is usually much larger than that of males. In the localities under our observation, the proportion of females to males among the captured adults (both A. maculipennis and A. punctipennis) is about two to one. This ratio does not, however, obtain among the insects bred from captured larvæ and pupæ; in any large series the numbers have been substantially equal. On this point our observations accord with those of Nuttall and Shipley, rather than with those of Rees.[2]

As a rule the larger larvæ develop into females and the smaller into males, as shown in the following records:

TABLE VII.

A. MACULIPENNIS.

Larval Length in mm.	Males	Females
7.5–8.0	0	12
7.0–7.5	8	10
6.0–7.0	5	0

These measurements were all made from freshly caught larvæ which pupated within four days after capture. From these facts the inference might seem legitimate that those larvæ which are supplied with abundant food, and consequently attain a larger size, develop into females, while those more scantily fed become males. It is quite possible, however, that other factors may enter into the problem. In every instance where a number of larvæ were bred from the egg under identical conditions in the same dish of water, the imagoes that appeared first were males and the later ones females. Rapidity of development and small size may be characters correlative with rather than determinative of sex. It seems clear that further observations are necessary.

[1] *Jour. of Hyg.*, 1901, 1, p. 452.

[2] *Jour. of Hyg.*, 1902, 2, p. 68.

LONGEVITY OF THE IMAGO.

Captured mosquitoes, as many observers have shown, feed readily upon fresh slices of banana and other fruit. Captured females almost invariably live longer than males. Ordinarily death occurs within two weeks. This we found to be true even among females confined under natural conditions in a large cage (3.5 ft. × 3.5 ft. × 5 ft.) of mosquito netting in the open. The cage was supplied with fresh water and fruit, and the mosquitoes were sometimes given a meal of blood. Occasionally under these conditions life was maintained for a month even in midsummer. At a lower temperature in the ice-box (10°) life may be considerably prolonged. In one case three captured males (A. maculipennis) were placed in the ice-box on July 22; one died twelve days later; the other two were still alive after fifty-four days. One female (A. punctipennis) survived several transfers to room temperature (60–70°) during transportation from place to place, and lived sixty-three days. The two species, A. maculipennis and A. punctipennis, showed no discernible difference in respect to their longevity.

BITING HABITS.

As a rule, A. maculipennis does not bite until at least twenty-four hours after hatching, and it is usually true that active females cannot be induced to make an attempt to draw blood until from two to three days after emergence. The first trials, moreover, are not always successful. Johnson[1] states that in no instance was he "able to induce Anopheles to bite until at least six days after leaving the pupa." Nuttall and Shipley,[2] on the other hand, affirm that they have seen A. maculipennis feed after twenty-four hours. Individual differences doubtless exist.

Females that have been kept in the ice-box will, when brought into a room, usually bite eagerly as often as every four or five days. On several occasions we observed females to bite after eggs had been laid. In one case a female A. punctipennis would not bite between August 23 and September 1. Then biting occurred on September 1, just after the laying of one set of eggs.

[1] *Loc. cit.*, p. 580. [2] *Jour. of Hyg.*, 1, p. 465.

A second set was laid on September 3 and a third on September 6, but this female refused to bite again and died in a few hours. After the females are enfeebled by egg-laying it may happen that although attempting to bite they fail to draw blood.

EGG-LAYING, ETC.

Captured females of A. maculipennis usually laid, if at all, within twenty-four hours to two weeks after being taken. One, however, laid only after thirty-nine days of captivity. In most cases masses of over 100 eggs were deposited. The largest number observed in any one laying was 230, and the smallest 7. Other layings were 140, 127, 153, 16, 214, 71, 27, 142, 153, 62, 63. The numbers range higher than those recorded by most European observers of this species. It often happened that captured females died after a few days' confinement without laying, although the abdomen was distended with eggs. Sometimes one female laid several sets of eggs at intervals of two or three days. This was observed more frequently in A. punctipennis than in A. maculipennis. The number of eggs laid by A. punctipennis averaged considerably less than the number laid by A. maculipennis. This may be a specific difference.

Eggs were laid by nine females (A. maculipennis) at a low temperature in the ice-box (10°). In several instances the number deposited was small, but in the majority of cases masses of eggs were obtained as large as at ordinary temperatures. This is a point worth noting in view of the resistance of the eggs to low temperature.

Eggs laid in the ice-box were kept at a low temperature for ten days; when placed at room temperature they hatched in about two days. Eggs that have begun to develop proceed slowly, even at 10°. One set laid at room temperature and allowed to remain for twenty-four hours before transfer to the ice-box hatched in the ice-box after a few days.

The females usually die within twenty-four hours after laying, particularly if the oviposition is complete, i. e., 100–200 eggs. Occasionally one may survive for several days; the maximum longevity observed after oviposition was seven days.

COLOR PREFERENCES OF A. PUNCTIPENNIS.

Experiments were made to determine the color preference of adult A. punctipennis. The conditions of the experiment were substantially the same as those in a similar series of observations made by Nuttall[1] upon A. maculipennis. The mosquitoes were confined under a large hood with glass sides and front. Boxes covered with different colored cloths of similar texture were placed under the hood, and every day at a fixed hour during a week the number of mosquitoes that had settled on each color was counted. The position of the boxes was changed every day after counting to eliminate possible influences of light and shade and other factors. The results were as follows:

TABLE VIII.

Number of Mosquitoes	On
61	Dark red
57	Dark blue
41	Black
8	Dark pink
6	Dark green
5	Lavender
5	Purple
4	White
2	Light blue
2	Pale green
1	Light pink
0	Yellow

These results with A. punctipennis are very much like those obtained by Nuttall with A. maculipennis, dark red and dark blue proving the most attractive in both cases.

EFFECT OF LOW TEMPERATURE.

Anopheles maculipennis eggs, larvæ and imagoes were subjected to temperatures below freezing (—5° to —10° C.), the cold being produced by mixtures of ice and salt.

a) Eggs that were frozen into a solid block of ice for one hour and then placed at room temperature hatched as usual on the third day after the experiment, only three hours later than control eggs which had remained constantly at room temperature. In another

[1] *Jour. of Hyg.*, 1902, 2, p. 74.

experiment, the low temperature (—5° to —10° C.) was maintained for twenty-four hours. When these eggs were thawed out and placed at the ordinary room temperature, they hatched only twenty-four hours later than the control.

b) Larvæ of assorted sizes frozen in the same way did not revive on thawing.

c) Imagoes placed in a dry test-tube and subjected to the same temperature (for one hour and for twenty-four hours) were rendered numb and inactive, but on being restored to room temperature, revived, were ready to bite, and lived for several days —in one case for eight days.

The considerable resistance to cold displayed by the eggs indicates the possibility of occasional hibernation in this stage.

SUMMER DIARRHEA IN INFANTS.*

George H. Weaver, R. M. Tunnicliff, P. G. Heinemann, May Michael.

In the spring of 1904, acting upon the suggestion of Dr. Bathena Coone, the management of the Chicago Daily News Sanitarium for sick babies requested the prosecution of some work directed toward the solution of some of the problems connected with the intestinal diseases in children. Funds for the purpose were furnished by the Sanitarium, while the Memorial Institute for Infectious Diseases and the Bacteriological Laboratory of the University of Chicago furnished the laboratory facilities.

It was believed that it would be especially valuable at this time to extend the work done last year under the direction of Dr. Flexner, with the object of learning whether the conditions in Chicago were identical with those in the eastern American cities. This seemed practically important, as physicians were being asked to try antidysenteric serum in the treatment of summer diarrhea.

In order to accomplish as much as possible in the short time during which summer diarrhea is prevalent, and in order to do it in the most satisfactory manner, various phases of the work were assigned to different persons. The clinical part of the study was left in the hands of Dr. Coone and Dr. Michael who were in attendance at the Sanitarium. Dr. Weaver and Dr. Tunnicliff undertook the bacterial examination of the stools, and the isolation and study of all bacilli which seemed to fall within the dysentery group. All cultures which corresponded culturally to the dysentery group of bacilli were sent to Mr. Heinemann, who undertook to test them as to their agglutinability with immune sera. The cultures were sent upon slants of agar, marked with numbers, but without intimation as to their cultural characters. Several known dysentery cultures were given him among the rest, marked also with simple numbers. It was hoped in this way to eliminate the personal factor as largely as possible.

The report of the work is here presented in three parts:

* Received for publication December 14, 1904.

I.

THE DYSENTERY BACILLUS GROUP IN DIARRHEAL DISEASES OF CHILDREN.

G. H. WEAVER AND R. M. TUNNICLIFF.

INTRODUCTION.

WHEN Shiga announced the discovery of the dysentery bacillus as the specific cause of epidemic dysentery in Japan, and Flexner in Manila, Kruse in Germany, Vedder and Duval in America, and other observers in various parts of the world found identical bacilli in cases of dysentery, the hope arose that the much-studied problem of the etiology of non-amebic dysentery had been solved. However, further study by improved methods of the bacilli isolated by these different workers has shown that bacilli which were said to be identical with the bacillus of Shiga were not always so in several physiological properties at least.

Flexner's bacillus, isolated in the Philippines, differs from the Shiga bacillus in its ability to break up carbohydrates and to produce indol. Jürgens in Europe has found bacilli in an epidemic of dysentery which correspond to the "Flexner-Harris" organism. In various epidemics of dysentery in this country Park and others have found bacilli corresponding to the true Shiga bacillus, and also the forms which differ from it in their effects upon carbohydrates.

Both Flexner and Park now recognize, not a single bacillus, but a group of bacilli, as causes of dysentery, and each has formulated a classification based upon the effects which the bacilli exert upon certain carbohydrates.* The bacilli included within these groups possess certain properties in common; i. e., inability to ferment glucose with liberation of gas, absence of motility, production of transient and faint acidity in litmus milk with subsequent return to an alkaline reaction. They are divided into two main groups

*Types of dysentery bacilli according to Park: (1) Shiga type. Do not ferment mannite, maltose, or saccharose; (2) Ferment mannite. Do not ferment maltose or saccharose. Produce indol; (3) Ferment mannite actively, also maltose (energetically), and saccharose (feebly). Produce indol.

Types of dysentery bacilli according to Flexner: (1) Shiga type: attacks glucose only; (2) Flexner-Harris type: attacks glucose, mannite, and dextrin, not lactose; (3) Bacillus "Y" type: attacks glucose and mannite, not dextrin and lactose.

by their ability or inability to break up *mannite* with formation of acid, and the former group is subdivided according to the action exhibited by the individuals upon maltose, saccharose, and dextrin.

Deycke[1] has described a bacillus, occurring in cases of severe dysentery in Constantinople, which differs again from any of these, in that it causes gaseous fermentation of glucose and acidifies milk even to a degree provoking coagulation. By feeding his cultures to cats he produced the anatomical lesions of dysentery. The experimental production of dysentery lesions in animals has also been reported as following the feeding and injection of other forms of dysentery bacilli.

The present tendency seems to be to enlarge the group of dysentery bacilli so as to make it include forms which differ quite widely from Shiga's bacillus as originally described. Our conception of dysentery at the present time is that of a clinical picture associated with considerable variation in the anatomical conditions, and due to any one of a group of bacilli which differ considerably among themselves. Our present position is much the same as before Shiga's discovery in that we must look upon dysentery as due to several different causes, although we know much more about a certain group of bacilli which probably bear an etiological relation to certain epidemics, and perhaps to sporadic cases. Before Shiga's time several investigators had apparently shown some relationship between certain organisms belonging in the group of colon bacilli and dysentery. The studies of Celli and Fiocca were especially valuable. They described Bacterium coli dysenteriæ as the essential cause of dysentery and they produced the disease in cats. This organism appears to be the same as that recently described by Deycke. Escherich, as early as 1886, in his pioneer work on intestinal bacteria in children, showed that colon bacilli and also B. lactis aerogenes produced hyperemia, swelling, and hemorrhage in the mucous membrane of the intestine in various animals when introduced subcutaneously or intravenously.

It seems that the poisonous products of many organisms, belonging to the colon and closely related groups of bacteria, are

[1] *Für die Türkei*, 1904, 2, p. 181.

able to give rise to marked disturbances in the intestinal mucosa in animals, corresponding more or less closely to the lesions found in human dysentery. In view of the fact that various forms of bacteria are associated with dysentery at different times, it seems not unreasonable to believe that each may cause the disease. Libman[1] stated this when he said: "One cannot help thinking it would be better to believe that dysentery may be due to a variety of organisms, although the disease is probably, in a great number of cases, in certain epidemics, due to one particular organism."

Since Shiga's publication, in which he laid stress upon the agglutinating power of the dysenteric patient's blood upon the specific organism, subsequent investigators have placed much reliance upon this phenomenon as an aid in the identification of dysentery bacilli. That too much dependence must not be attached to the agglutination reaction as a means of diagnosis of a disease, or for the identification of the specific cause of a disease must be acknowledged. The agglutination reaction in typhoid fever has lost much of its specific interpretation as a result of more extensive and comprehensive investigation.

Durham[2] performed many tests to determine whether it was possible to differentiate various organisms of the colon-typhoid group by agglutinative serum reactions, and concluded that "it is clear that the clumping reaction is of little value for differentiating and classifying these bacilli in a satisfactory manner." Stober,[3] working upon the agglutination of typhoid and paratyphoid bacilli by various immune sera, found that in certain agglutinations it was necessary to remove the common agglutinins by saturation methods, before anything final could be arrived at concerning specific agglutinins for typhoid and paratyphoid bacilli. In a study of agglutination in the group of fluorescent bacteria, Lincoln[4] found no definite relation between the biological characteristics and the agglutinative reaction. Park and Collins[5] found it necessary to remove the "normal agglutinins" or "common agglutinins" from an immune serum

[1] *Jour. Amer. Med. Assn.*, 1904, 43, p. 383.
[2] *Jour. of Exper. Med.*, 1901, 5, p. 353.
[3] *Jour. of Infect. Dis.*, 1904, 1, p. 445.
[4] *Ibid.*, 1904, 1, p. 268.
[5] *Proc. N. Y. Path. Soc.*, 1904, 3, p. 213.

in order to obtain a specific agglutinating serum for the dysentery organism injected.

Stimulated by the successful results obtained through bacteriological studies of dysentery, Duval and Bassett[1] in the summer of 1902 undertook by the same methods a study of summer diarrhea in infants, availing themselves of the agglutinating power of the blood of the patients upon the bacteria isolated to determine the relationship between the bacteria and the disease. They studied 53 cases, and in the stools of 42 the bacillus of dysentery was found, 11 cases giving negative results. Agglutination reactions were obtained when the organisms were tested: (*a*) with the blood serum of the patients from whom they were secured; (*b*) with the serum of other infants suffering from summer diarrhea; (*c*) with the serum of adult patients with acute dysentery; (*d*) with anti-dysenteric, immune serum. The specific bacillus was not found in the stools of 25 healthy children, nor of those suffering from simple diarrhea, marasmus, and malnutrition; nor did the blood serum of these latter individuals agglutinate the dysentery bacillus. The cases from which the dysentery bacillus was isolated included examples of so-called dyspeptic diarrhea, of enterocolitis, and of malnutrition and marasmus with superimposed infection. They believed that their findings justified them in the conclusion that the summer diarrheas of children are caused by intestinal infection with Bacillus dysenteriæ Shiga, and therefore are etiologically identical with the acute bacillary dysentery of adults.

The following winter Wollstein[2] made a bacteriological study of 114 cases of diarrhea in infants and children occurring in New York city. The dysentery bacillus was found in 39. The agglutinating power of the patient's blood upon dysentery bacilli was tested, and a good reaction obtained in 21 cases. The important observation was made that the reaction was obtained in seven cases, only after injection of "Harris" serum.

During the summer of 1903 extensive bacteriological studies of the diarrheal diseases of children were undertaken by the

[1] *Amer. Med.*, Sept. 13, 1902, 4, p. 417.

[2] *Jour. of Med. Research*, 1903, 10, p. 11.

Rockefeller Institute for Medical Research[1] under the direction of Dr. Flexner. In this collective investigation 412 cases of diarrheal disease among children were studied with reference to the presence of the bacillus of dysentery. Positive results were obtained in 279, or 63.2 per cent. The cases were mostly unselected, and the examinations were carried out by nine different investigators or pairs of investigators in Boston, Baltimore, New York, and Philadelphia. The proportion of cases yielding dysentery bacilli varied much with different investigators, and was from 40 to 94 per cent. About one-third of the positive results were obtained in the examination of stools containing blood, and in all cases yielding positive results mucus was present in the stools, with the exception of three cases in which the stools were entirely fecal with no blood or mucus. In 23 cases the Shiga type of bacillus was alone cultivated, in six it was associated with the "Flexner-Harris" type, and in the others the "Flexner-Harris" type was found alone. All the *mannite* fermenters were classed as examples of the "Flexner-Harris" type. Duval and Shorer, however, found 12 examples of the Bacillus "Y" of Hiss and Russell among their "Flexner-Harris" cultures.

In the course of these investigations Duval[2] encountered a form of bacillus, which differs radically from the "Shiga" and "Flexner-Harris" types, and which he places in the group of dysentery bacilli. It produces a primary acidity in litmus milk, which is replaced by an alkaline reaction after 48 hours, and after a second period of five or six days a second acidity, exceeding the primary in intensity, makes its appearance and is permanent. Flexner classifies this new type as "Bacillus A" and "Bacillus B;" the former attacking glucose, *mannite* and lactose, but not dextrin; the latter attacking dextrin as well as the others. He, however, holds that this type demands additional study before admission to the group. It would appear to approach more nearly to the group of colon bacilli. In the course of these studies it was found that the blood of children suffering from diarrheal disease agglutinates at time the bacillus of dysentery in

[1] *Studies from the Rockefeller Institute for Medical Research*, 1904, 2, p. 7.

[2] *Ibid.*, p. 42, and *Jour. Amer. Med. Assn.*, 1904, 43, p. 381.

"high dilution" (1:20 to 1:1500), but that it could not be treated as an index of the presence of, or infection with Bacillus dysenteriæ. In the negative cases, i. e., those in which no dysentery bacilli were found, the agglutination reaction was often obtained by Bassett at the same dilutions. Search was made for the dysentery bacillus in the stools of healthy children by Wollstein and Duval, and the latter isolated a small number of dysentery bacilli from the stools of two healthy, milk-fed children. These bacilli gave all the reactions of the "Flexnêr-Harris" bacillus.

During the summer of 1903 Dunn[1] examined 61 cases of summer diarrhea, and found the dysentery bacillus in 10. Organisms were found in other cases, which corresponded culturally to dysentery bacilli, but they were excluded because agglutination reactions were unsatisfactory.

Park, Collins, and Goodwin[2] reported the examination of the stools from a large number of cases of summer diarrhea with excessive mucus, with or without blood. In a majority of the cases the *mannite* fermenting type of dysentery bacillus was found, although at times in small numbers. In none was the Shiga bacillus discovered. In cases of cholera infantum, no dysentery bacilli of either type were found. The blood from cases of cholera infantum and simple diarrhea did not agglutinate any of the dysentery bacilli in their collection higher than 1:10.

Schwartz[3] examined 30 cases of typical summer diarrhea with profuse watery stools and little or no mucus. He did not find dysentery bacilli in any. The blood of such cases did not agglutinate dysentery bacilli.

BACTERIAL EXAMINATION OF THE CONTENTS OF THE LOWER PART OF THE INTESTINE IN CASES OF SUMMER DIARRHEA.*

As there were no laboratory facilities at the Sanitarium, it was necessary to carry the material for cultures to the Laboratory of the Memorial Institute for Infectious Diseases. In order that the material might be collected and transported without danger

[1] *Amer. Med.*, 1904, 7, p. 737. [2] *Proc. N. Y. Path. Soc.*, 1903, 3, p. 148.

[3] *Proc. N. Y. Path. Soc.*, 1903, 3, p. 172.

*The authors desire to express their thanks to Dr. Alice Hamilton for help in working out some of the earlier cases.

of contamination, a simple piece of apparatus was devised. An eight-ounce, wide-mouthed bottle was supplied with a tight-fitting rubber stopper having two perforations. Through one perforation was passed a piece of glass tubing, reaching three centimeters above the stopper, and half-way to the bottom of the bottle. This was closed by a cotton plug at the outer end. A second glass tube was passed just through the stopper in the other perforation, and connected without to a piece of rubber tubing 50 cm. long. By means of a short piece of glass tubing, the distal end of this rubber tubing was connected with a No. 12 or 14 soft rubber catheter. The distal 25 cm. of the catheter was covered by a piece of soft rubber tubing, large enough to slide freely over the catheter, and closed at the ends with cotton. In the bottle was placed two to three ounces of 0.85% solution of sodium chloride. The entire apparatus was then sterilized in the autoclave, and before removal, the rubber stopper was forced tightly into the bottle. If any of the cotton plugs had become wet during the sterilization, they were carefully replaced by dry, sterile cotton.

A number of such sterile outfits were carried to the Sanitarium, and the material obtained as follows: The cotton plugging having been removed from the rubber shield covering the catheter, the free end of the catheter was pushed a little beyond the end of the shield, and some sterile vaseline rubbed over it with a sterile applicator. The bottle was then inverted and elevated by an assistant, care being taken that no fluid entered the air tube, and the salt solution allowed to fill the tube and catheter. The further flow being prevented by pinching the tube near the bottle, the catheter was carefully introduced into the rectum. The manipulations were all carried out by means of the shield, the hands never touching the part of the catheter which entered the rectum. When the catheter had been introduced from 10 to 12 cm., the salt solution was allowed to run out through the catheter, the flow being stopped by pressure upon the rubber tubing while the tube and catheter were still filled with the solution. The bottle was now turned upright and lowered to a position considerably below that of the child's body, and part or all of the salt solution

allowed to flow back into the bottle carrying with it some intestinal contents. When the stools contained mucus, some of it was readily obtained in this way, quite often large masses escaping into the bottle. After withdrawal of the catheter, the rubber stopper was at once replaced by one of sterile cotton. The bottles with the samples were then taken directly to the laboratory, the transportation requiring about one hour, and cultures were prepared at once.

This method of collecting the intestinal contents was devised to meet the demands of our peculiar position. It, however, possesses certain advantages over making cultures from diapers. The material is less apt to contain the organisms which multiply especially in the rectum (B. alcaligenes, B. pseudodysentericus Müller), and contamination from without is impossible.

In making the cultures the methods elaborated by Duval and Bassett were employed. When mucus was present in the salt solution, some was removed, washed in sterile broth once or twice, and then broken up in a tube of sterile broth, and plates prepared from it. Pieces of mucus with blood were chosen when present. In the specimens from which no mucus could be obtained, and in the ones with mucus after some of it had been removed, the fluid was thoroughly agitated, and after standing long enough for the coarse particles to settle, some of the upper turbid fluid was removed with sterile pipettes, and plate cultures prepared from it.* The plates were made from nutrient peptone-agar with a reaction of 1.5% acid to phenolphthalein. From 8 to 16 plate cultures were made from each specimen, and when mucus was obtained as many plate cultures were prepared from it also. The plates were placed in the incubator in an inverted position, and after 18 hours the colonies which had grown out in the suitable plates were marked by a circle with a wax pencil. The plates were then returned to the incubator, and allowed to remain 24 hours longer. From the colonies most resembling dysentery bacilli, stab inoculations were made into glucose-agar. The

*Sometimes the cultures prepared directly from particles of mucus yielded much more numerous bacilli resembling dysentery bacilli than did cultures from the salt solution. As often, however, the opposite was true, and there did not seem to be any particular advantage in making cultures from the washed mucus only.

colonies which grew out in the second 24 hours were selected by preference, but some of the more delicate ones among those growing out in 18 hours were also studied.

The cultures which did not form gas in glucose-agar after two days in the incubator were reserved for further study, the others being discarded. Litmus milk was inoculated from the non-gas-forming cultures. Those which did not produce a strong acidity in three days were studied fully as to their cultural properties, and effects upon carbohydrates. All the cultures which corresponded to the dysentery bacillus group were sent to Mr. Heinemann for agglutination tests.

Cultures were made and studied from 102 cases.* From 76 cases no bacilli corresponding to any of the types included in the dysentery bacillus group were found. From these cases a total of 2757 colonies were studied. From 26 cases (25.4 per cent.) bacilli were cultivated which possess the cultural properties shown by one or the other member of the dysentery bacillus group.

The appended tables show the cases from which cultures were prepared and the number of colonies studied from the plates in each case.

In Table I is given a list of the cases from which no bacilli resembling the dysentery bacillus group were obtained.

Table II shows the principal cultural and physiological properties of the cultures which corresponded to the dysentery group. It also gives the results of Mr. Heinemann's agglutination tests upon these cultures. A few known cultures are included for comparison.

All the cultures included in this table possessed the following common characteristics: Morphologically they were short rods, about as long as typhoid bacilli, but thicker and plumper with rounded ends. Threads of two to six members were occasionally observed. In the same culture, shorter individuals were sometimes seen among the longer ones. In glucose-agar involution forms were often present. No considerable difference could be observed among the various cultures. None of them stained by

* Ninety-eight of the cases were in the Daily News Sanitarium. Cases A, B, and C were in the Presbyterian and Cook County Hospital, and for the opportunity to study them we are under obligation to Dr. A. C. Cotton and Dr. Wm. J. Butler.

Gram's method. No efforts were made to stain flagella. In broth after 24 hours there was a dense cloudiness, some bacilli beginning to settle to the bottom. Later the precipitate was abundant, the upper fluid becoming clear. On the agar slant the growth was delicate, pearly, not elevated, spreading but little, with even or slightly irregular edges. There occurred a cloudiness in the water of condensation with rapid precipitation of the growth to the bottom. On the surface of the gelatin stab culture they spread out two to three millimeters as a semitransparent, typhoid-like growth. A continuous filament developed along the stab. There was no liquefaction. No gas was formed in glucose-agar. They rendered litmus milk slightly acid in 24 hours, and on the third day the original color was restored and was permanent for a month. They all grew as non-motile organisms in Hiss's semisolid medium.

It will be observed that no culture was obtained which corresponded to the true Shiga bacillus. Grouped according to the classification of Flexner, 15 cultures from 11 cases corresponded to the "Flexner-Harris" type, and 18 cultures from 17 cases to the "Bacillus Y" type. From two cases both these types were obtained.

When we attempt to group these cultures according to the classification of Park, there remains a group which is not provided for, which ferments saccharose and not maltose. The agglutination reactions do not bear any relationship to the cultural properties in many cases.

It is of interest to note a peculiar behavior exhibited by several cultures as regards their effects upon sugars. Some of the cultures immediately after isolation failed to ferment maltose or saccharose or dextrin, and after cultivation for several generations they were able to ferment one or more of the sugars which they were not able to attack at first. In a few instances the opposite was true, the ability to ferment one of these sugars being lost. The results given in the table were obtained with the cultures after they had been cultivated for several generations upon artificial media. Flexner has said that "the manner in which this group of organisms agrees and differs is of interest

and importance, and cannot fail to arouse the suspicion that their physiological properties are at present in a very unstable condition." It would appear from the observations here recorded, that the effects of these organisms upon maltose, saccharose and dextrin are not constant but may vary according to the conditions in which the organisms have been growing.

If we attempt to decide which of our cultures were true dysentery bacilli from the agglutinating effects of immune sera upon them, we shall be at a loss to know which should be included and which not. Because of the absence of agglutination, several of the cultures would be classed as Bacillus pseudodysentericus Müller. Ford[1] has found this organism in 10 out of 50 cases in which he studied the intestinal flora. He found it most often in the rectum. None of the cases from which he cultivated it were cases of dysentery. This bacillus possessed all the cultural and morphological features of B. dysenteriæ, and it was differentiated only by its failure to give characteristic serum reactions.

When we try to arrive at any conclusions regarding the relationship existing between the dysentery bacillus and cases of summer diarrhea in children, we were met by certain difficulties. In the first place, which of the bacteria cultivated from stools and corresponding more or less closely to the original cultures of dysentery bacilli, shall be called dysentery bacilli? Are we to depend upon the agglutination of the bacilli by immune serum as the final test, and if so are we to employ a serum in each case prepared with a culture similar to the one to be tested?

A second difficulty is dependent upon the fact that however many colonies are studied from the plates prepared from a case, the failure to find the bacilli sought does not exclude the possibility that they were present in such numbers as to have been missed, and that they might have been found if several hundred or more colonies had been studied.

However the case may be, in epidemic dysentery in children, it looks as if we should be obliged for the present to say that we are unacquainted with a specific cause for all cases of summer diarrhea in infants, but that it is likely that many cases are due

[1] *Studies from the Royal Victoria Hospital*, 1903, 1, No. 5.

to the single or combined action of various forms of bacteria. Booker[1] long ago pointed out the fact that in infants affected with summer diarrhea, the inconstant varieties of intestinal bacteria are much more prominent and frequently appear in immense numbers. Alterations in the chemical composition of the intestinal contents dependent upon disturbances in digestion from improper feeding, high temperature, etc., may furnish favorable conditions for the growth of various bacteria which are present in the intestine more or less constantly in small numbers, and on the other hand conditions which are inhibitory to the growth of the bacteria which are present in largest numbers in health. The presence of a certain bacterium in large numbers does not determine its etiological connection with the disease.

The degree of agglutinability is so variable in the case of certain well-known bacteria (as the typhoid bacillus), varying at different times even in the same culture, that it is unsafe to build too much upon such reactions in certain imperfectly studied cultures for purposes of diagnosis.

A limited number of tests were made by us to determine if possible the extent to which the agglutination of dysentery bacilli by the blood of children suffering from summer diarrhea could be considered specific. These tests were made by the microscopic method as the amount of blood serum obtained was small. The results are given in Table III, and they appear to show that agglutinins for the dysentery group of bacilli may be formed in the course of infectious diseases having no possible connection with dysenteric or diarrheal diseases.

In two of the cases of diarrhea from which we obtained blood for agglutination tests, a previous injection of antidysenteric serum had been administered. The serum from these cases agglutinated at higher dilution than in the others. Wollstein had previously noted that agglutinins appear in the blood after the injection of antidysenteric serum. When agglutination tests in summer diarrhea are reported, it is important to know whether antidysenteric serum has been given previously, as the degree of

[1] *Johns Hopkins Hosp. Rpts.*, 1897, 6, p. 251.

agglutination may probably be very much increased in this manner. In view of the presence of dysentery bacilli in normal milk stools, and their absence or presence in small numbers in the stools of a large proportion of children with summer diarrhea, it is not safe to assume that their presence is connected causally with the majority of cases of summer diarrhea.

TABLE I.

CASES FROM WHICH NO CULTURES RESEMBLING THE DYSENTERY BACILLUS GROUP WERE OBTAINED.

CASE	NO. OF COLONIES STUDIED FROM PLATES		CASE	NO. OF COLONIES STUDIED FROM PLATES	
	Forming Gas	Not Forming Gas		Forming Gas	Not Forming Gas
1	28	0	55	10	16
2	23	1	57	23	12
3	19	0	59	16	2
4	25	9	60	39	1
5	33	2	61	15	8
6	33	5	62	28	8
7	0	16	63	42	0
9	55	4	64	43	0
10	52	3	65	33	0
12	71	0	77	21	12
13	52	0	79	7	25
14	24	0	80	0	34
16	24	0	81	1	13
18	21	3	84	36	0
20	71	0	85	36	0
21	46	2	87	9	14
22	31	0	88	25	0
24	53	3	89	21	2
25	36	0	90	9	15
26	130	2	91	24	0
28	24	10	92	24	0
29	36	0	97	72	0
30	34	1	98	36	0
31	25	11	99	23	1
32	48	0	100	35	1
33	31	4	104	50	0
34	24	0	105	50	0
35	5	19	107	1	35
38	9	0	108	48	0
40	34	4	111	35	1
41	23	12	113	0	14
42	1	33	114	22	0
44	32	1	116	22	15
45	28	1	C	65	1
47	35	1	D	36	0
48	29	7			
49	36	0	Total	2368	389
50	36	0			2368
51	35	1			
52	37	1			
53	22	3	Grand total		2757

TABLE II.

CULTURES BELONGING IN THE DYSENTERY BACILLUS GROUP.

CASE	CULTURE	NO. OF COLONIES STUDIED FROM PLATES		INDOL		NUTROSE-LITMUS[1] SOLUTIONS + 1% CARBOHYDRATES (Results after 14 Days)								AGGLUTINATION BY IMMUNE RABBIT'S SERUM (Numbers Represent Dilutions at Which Reaction Occurred)				TYPES (FLEXNER)	TYPES (PARK)
		No. Corresponding to Description	No. not Resembling Dysentery Bacillus Culturally	5 Days	9 Days	Mannit	Maltose	Saccharose	Dextrin	Lactose	Dextrose	Galactose	Levulose	Shiga	Kruse	Flexner	"Y" Hiss		
11	6	2	132	?	0	1	0	0	0	0	C	C	C	...	...	...	...	3	II
15	1	10	14	+	++	3	0	0	0	0	C	3	..	...	100	...	100	3	II
17	1	32	0	?	0	3	0	0	0	0	C	3	..	...	50	200	100	3	II
19	1	59	9	+	+	3	C	1	1	0	C	C	C	...	...	100	...	2	III
19	30	1	..	+	++	3	3	0	2	0	C	C	..	100	50	200	...	2	III
27	1	1	79	+	0	3	3	1	1	0	C	C	..	...	...	100	100	2	III
36	1	13	5	+	+	3	0	1	0	0	3	3	..	...	...	100	...	3	II
36(2)	1	4	20	+	+	3	0	1	0	0	C	C	C	...	200	...	50	3	..
37	1	1	23	?	0	1	0	0	0	0	C	C	C	100	...	...	100	3	II
39	1	7	8	+	+++	3	3	3	0	0	C	3	..	200	...	200	...	3	III
43	5	6	11	...	+	3	0	C	0	0	C	3	..	...	...	...	...	3	...
54	3	3	..	...	+	3	0	0	0	0	C	3	..	...	...	100	200	3	II
55	11	3	24	+	+	3	0	3	0	0	C	C	..	200	100	200	200	3	...
56	1	4	18	+	?	3	0	0	0	0	3	3	..	200	...	50	200	3	II
76	3	15	14	?	+++	3	0	0	2	0	C	C	..	...	50	...	100	2	II
78	1	4	16	?	+	3	3	0	0	0	C	3	..	200	...	200	...	2	III
82	1	1	35	?	?	2	3	0	3	0	C	C	C	200	100	200	100	2	III
83	1	2	34	0	0	3	2	1	1	0	C	C	C	100	100	...	100	2	III
86	1	35	1	+	++	3	0	1	0	0	C	C	C	50	...	...	100	3	...
93	1	3	32	+	+	C	2	1	1	0	C	C	C	...	...	...	100	2	III
93	3	1	..	+	+++	3	1	1	1	0	C	C	C	...	...	...	...	2	III
96	1	1	35	+	0	3	C	1	2	0	C	C	C	...	50	...	...	2	III
101	1	6	44	0	+	3	3	1	1	0	C	C	C	200	...	...	...	2	III
101	5	1	..	0	0	3	0	2	0	0	C	C	C	200	...	...	...	3	...
102	1	4	50	?	?	1	3	0	3	0	C	C	C	50	200	50	50	2	III
102	2	..	50	++	+++	1	C	0	1	0	C	C	C	..	...	200	...	2	III
102	6	..	50	++	+++	1	C	0	3	0	C	C	C	100	...	200	...	2	III

TABLE II—*Continued.*

CASE	CULTURE	NO. OF COLONIES STUDIED FROM PLATES		INDOL		NUTROSE-LITMUS[1] SOLUTIONS + 1% CARBOHYDRATES (Results after 14 Days)								AGGLUTINATION BY IMMUNE RABBIT'S SERUM (Numbers Represent Dilutions at Which Reaction Occurred)				TYPES (FLEXNER)	TYPES (PARK)
		No. Corresponding to Description	No. not Resembling Dysentery Bacillus Culturally	5 Days	9 Days	Mannit	Maltose	Saccharose	Dextrin	Lactose	Dextrose	Galactose	Levulose	Shiga	Kruse	Flexner	"Y" Hiss		
103	7	1	35	++	++	C	0	2	0	0	3	C	C	...	50	...	50	3	...
109	4	4	32	+	+	2	0	0	0	0	C	C	..	100	...	...	...	3	II
110	1	40	28	+	+	3	0	0	0	0	C	C	..	100	...	200	200	3	II
A	1	34	2	+	++	C	C	0	1	0	C	3	C	...	...	...	...	2	III
B	1	10	1	?	0	3	C	2	3	0	C	3	C	...	...	200	...	2	III
B	4	25	1	....	+	3	0	2	0	0	C	3	C	...	...	200	200	3	...
Known Cultures[2]—																			
B. Dys.—Shiga	..	...		0	?	0	0	0	0	0	C	C	C	100	200	...	...	1	I
B. Dys.—Kruse	..	...		?	0	0	0	0	0	0	C	C	C	100	200	...	...	1	I
B. Dys.—Flexner	..	...		+++	+++	3	C	1	3	0	C	C	C	...	...	200	200	2	III
B. Dys.—Mt. Vernon CC$_2$	..	...		....	...	2	0	0	0	0	C	C	C	...	...	100	200	3	II
B. Dys.—N. Y. city—Salaut	..	...		+++	+++	2	2	1	0	0	3	C	C	...	...	...	...	3	III
B. Dys.—Coney Isl.—Etieme	..	...		+	+	1	3	2	0	0	3	1	..	...	...	...	...	3	III
B. Dys.—Mt. Desert—Park	..	...		+	+	2	0	0	0	0	3	C	..	200	...	...	...	3	II
B. Dys.—"Y" Hiss & Russell	..	...		+	+	2	0	0	0	0	C	C	..	200	100	...	200	3	II

[1] All the cultures in nutrose-litmus solutions were made at the same time, and from the same lot of medium. These same cultures in the same nutrose-litmus solution without the addition of any carbohydrate failed to bring about any alteration in color. The sugars added were Merck's chemically pure. "1," "2," and "3" indicate changes in the color of the litmus due to acid production; from the slightest perceptible reddening ("1") to a strong acid reaction ("3"). "C" signified a pecipitation of the nutrose.

[2] For the first three and the last of these cultures, we are under obligation to Professor Jordan, of the University of Chicago; for the remainder to Dr. Collins in Dr. Park's laboratory in New York city.

TABLE III.

MICROSCOPIC AGGLUTINATION OF BACILLI OF THE DYSENTERY GROUP BY THE SERA OF CHILDREN.*

SOURCES OF SERA	CULTURES. FIGURES REPRESENT DEGREES OF DILUTION							
	19 – 1	B. Dys. Mt. Desert Park	B. Dys. Shiga	B. Dys. Flexner	B. Dys. N. Y. City; Salant	B. Dys. "Y" Hiss and Russell	A – 1	B – 1
Case 19: Entero-colitis. Dys. bacilli cultivated. Had received antidysenteric serum.	1:800=+	1:50 =+	1:50 =0	1:100=+	.....	.....	.....	.....
Case A: Entero-colitis. Dys. bacilli cultivated. Had received antidysenteric serum.	1:20 =+	1:100=+	1:20 =+	1:100=+	1:400=+	1:100=+	1:400=+	.....
Case 71: Entero-colitis. No bacteriologic examination.	1:20 =0	1:20 =0	1:20 =+	1:20 =+	1:20 =+	1:20 =0	1:20 =+	.....
Case 74: Entero-colitis. No bacteriologic examination.	1:20 =+	1:20 =+	1:20 =+	1:20 =+	1:100=+	1:20 =+	1:100=+	.....
Case 113: Entero-colitis. No Dys. bacilli found in cultures.	1:100=+	1:50 =0	1:50 =0	1:50 =0	1:50 =0	1:50 =0	1:50 =0	.....
Case 116: Entero-colitis. No Dys. bacilli found in cultures.	1:20 =0	1:20 =0	1:20 =0	1:20 =0	1:20 =0	1:20 =0	1:20 =+	.....
Case B: Entero-colitis. Dys. bacilli cultivated.	1:50 =+	1:50 =+	1:20 =0	1:100=+	1:50 =+	1:50 =+	1:20 =+	1:200=+
Case C: Entero-colitis. No Dys. bacilli found in cultures.	1:20 =0	1:20 =0	1:20 =0	1:50 =+	1:50 =+	1:20 =+	1:20 =0	1:50 =+
Scarlet-fever—Case 378	1:200=+	1:20 =0	1:20 =0	1:100=+	1:50 =+	1:20 =0	1:20 =+	1:200=+
Scarlet-fever—Case 379	1:100=+	1:100=+	1:20 =+	1:100=+	1:100=+	1:50 =+	1:50 =+	1:200=+
Scarlet-fever—Case 380	1:20 =0	1:20 =0	1:20 =0	1:20 =0	1:50 =0	1:20 =0	1:20 =0	1:20 =0
Typhoid-fever—convalescent—E. E.	1:50 =+	1:50 =0	1:50 =0	1:50 =+	1:50 =0	1:50 =0	1:50 =0	1:50 =0
Typhoid-fever—convalescent—P. E.	1:50 =+	1:100=+	1:50 =+	1:100=+	1:100=+	1:200=+	1:50 =0	1:200=+
Typhoid-fever—convalescent—R. H.	1:50 =+	1:50 =+	1:50 =0	1:100=+	1:50 =+	1:50 =+	1:50 =0	1:100=+
Measles—fifth day—P. J.	1:100=+	1:100=0	1:100=0	1:100=+	1:100=+	1:100=0	1:100=+	.....
Measles—fifth day—M. L.	1:100=+	1:100=0	1:100=0	1:100=+	1:100=+	1:100=0	1:100=0	.....

*The dilution at which a negative result is recorded was the lowest dilution tested; and, with a few exceptions, where positive results were recorded, it was determined that agglutination did not occur at a dilution twice as great.

The character of the cultures will be found in Table II.

II.

STUDY OF THE AGGLUTINATING POWER OF IMMUNE RABBIT SERA UPON 33 CULTURES OF THE DYSENTERY BACILLUS GROUP, ISOLATED FROM CASES OF SUMMER DIARRHEA.*

P. G. HEINEMANN.

THE methods employed in testing the agglutinative properties of organisms obtained from infantile diarrhea may be briefly summed up as follows:

Four different strains of dysentery bacilli were obtained from the laboratory collection known as:

1) Shiga type.[1]
2) Kruse type.[2]
3) Flexner ("Gray" Manila) type.[3]
4) The organism described by Hiss and Russell as type "Y."[4]

These four organisms were sent to the University of Chicago laboratory by Dr. P. H. Hiss of Columbia University, and were received on May 9, 1903. Subcultures had been made from the original cultures at regular intervals.

Eight healthy rabbits were selected and injected subcutaneously, two always with the same type. Only two survived longer than a few weeks, in consequence of which fact the agglutinating power of the serum never exceeded a dilution of 1:200. This, however, may not have been a serious disadvantage. Bergey[5] states, that a high immunization of an animal against a particular organism not only increases the agglutinin for that organism, but also induces an augmentation of the agglutinins of other organisms of closely allied species. This principle seems especially applicable to the different strains of dysentery organisms, as several authors have shown that sera of animals immunized with one strain will agglutinate others in fairly high dilutions. Jürgens[6]

*The work was done at the Bacteriological Laboratory at the University of Chicago under the direction of Professor E. O. Jordan, and I take this opportunity of acknowledging my indebtedness for his kind interest and advice.

[1] *Centralbl. f. Bakt.*, 1898, 23, p. 599.
[2] *Deutsche med. Wchnschr.*, 1900, 26, p. 637.
[3] *Johns Hopkins Hosp. Bull.*, 1900, 11, p. 23.
[4] *Med. News*, Feb. 14, 1903, 82, p. 82.
[5] *Jour. Med. Research*, 1903, 10, p. 21.
[6] *Ztschr. f. klin. Med.*, 1904, 51, p. 365.

has shown that the serum of a patient, which agglutinates the "Kruse" type, will also agglutinate the "Flexner" type and calls this phenomenon "Group agglutination."

In a recent paper[1] Dr. Park describes a number of experiments and states that specific agglutinins develop in the early period of treatment far in excess of group agglutinins. At later periods group agglutinins develop with greater rapidity than specific agglutinins, often constituting 50% of the total amount of agglutinins present. At still later periods of treatment specific agglutinins diminish with greater rapidity than group agglutinins.

Injections were given regularly twice a week, with a few exceptions, so as to enable the animals to fully recover from the effects. The initial amount injected was 0.5 c.c. of a 24-hour broth culture. The Shiga cultures seemed the most fatal of the four strains, the rabbits sometimes dying after two or three injections, ten rabbits having succumbed apparently directly from dysentery bacillus infection. Martini and Lentz[2] report a similar experience in regard to rabbits and guinea pigs. The other three strains were fatal to two rabbits each, two additional ones having died from different infections. There can be little doubt that the summer weather contributed toward the death of all these animals.

After such experiences, the new rabbits were treated with smaller doses, commencing with 0.25 c.c. of the 24-hour broth culture. Later, agar slant cultures were used exclusively. The growth was scraped off by means of a platinum loop, suspended in 4 c.c. 0.85% NaCl solution and this suspension exposed to a temperature of 65° C. for 30 minutes. This mode of procedure seemed more successful, proving to be less fatal to the animals as well as producing an agglutinating power of the blood serum in a shorter time. This method was continued to the end, with increasing doses as shown in Table IV.

The rabbits, immunized to the same strain, were bled alternately, unless the death of one of the pair made it necessary to use the same animal twice in succession. The blood obtained was

[1] *Jour. of Med. Research*, 1904, 12, p. 491.

[2] *Ztschr. f. Hyg. u. Infektionskr.*, 1902, 41, p. 540.

TABLE IV.

DATA RELATING TO INJECTION OF ANIMALS.

DATE	MATERIAL INJECTED	SHIGA-BACILLUS								KRUSE-BACILLUS						FLEXNER-BACILLUS				"Y"-BACILLUS			
		Amount Injected into Rabbit No.								Amount Injected into Rabbit No.						Amount Injected into Rabbit No.				Amount Injected into Rabbit No.			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	Broth	c.c.	c.c.	c.c.	c.c.	c.c.		c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c c.	c.c.
June 28..	Cultures	0.5	0.5							0.5	0.5					0.5	0.5			0.5	0.5		
July 1..	"	0.5	0.5							0.5	0.5					0.5	0.5			0.5	0.5		
July 6..	"	0.5	dead							0.5	0.5					0.5	0.5			0.5	0.5		
July 9..	"	0.5		0.5						dead	0.5					0.5	0.5			0.5	0.5		
July 12..	"	0.5		dead							0.5	0.5				0.5	dead			0.5	0.5		
July 15..	"				0.25						dead	0.5	0.25					0.25					
July 19..	"	0.5			dead	0.25						0.5	0.5			0.5		0.25		0.5	0.5		
July 22..	"	0.5				dead						0.5	0.5			0.5		0.25		0.5	0.5		
	Agar Cul's.																						
July 25..	killed at 65°	1.					0.5					1.	1.			1.		1.		1.	1.		
July 30..	"	1.					1.					1.	1			1.		1.		1.	dead		
Aug. 3..	"	1.5					1.5					1.5	dead	1.		1.5		1.5		1.5		1.	
Aug. 6..	"	1.5					1.5					1.5		1.		1.5		1.5		1.5		dead	0.5
Aug. 10..	"	1.5					1.5					1.5		1.5		1.5		1.5		1.5			1.
Aug. 19..	"	dead					1.5	1.				1.5		1.5		1.5		1.5		1.5			1.5
Aug. 24..	"						1.5	1.				1.5		1.5		1.5		1.5		2.			1.5
Aug. 29..	"						1.5	1.				1.5		1.5		1.5		dead	0.5	2.			1.5
Sept. 2..	"						1.5					1.5		1.5		1.5			0.5	2.			1.5
Sept. 14..	"						1.5	dead	0.5			dead		1.5	0.5	1.5			1.	2.			1.5
Sept. 17..	"						1.5		1.					1.5	1.	1.5			1.	2.			1.5
Sept. 21..	"						1.5		1.5					1.5	1.5	1.5			1.5	2.			1.5
Sept. 24..	"						1.5		1.5					1.5	1.5	1.5			1.5	2.			1.5
Total....	.....	10.0	1.0	0.5	0.25	0.25	18.0	3.0	4.5	1.5	2.5	14.5	3.25	15.5	4.5	22.0	2.0	10.25	6.0	25.5	4.5	1.0	13.5

left in the ice-chest over night, the serum collected the following morning, and if necessary filtered through sterile absorbent cotton and then mixed with a suspension of the different organisms under investigation.

These organisms were received at the laboratory cultivated on agar slants, the tubes being numbered, without any indication whatever as to their properties. Subcultures on agar slants were prepared and incubated at 37° C. for 24 hours.

The agglutination tests were made by the macroscopic method exclusively. The 24-hour-old agar growth was scraped off, shaken and diluted with a sufficient amount of 0.85 % NaCl solution to obtain a light and uniform degree of cloudiness. This suspension was then filtered through absorbent cotton, often repeatedly, until perfectly free from flakes and until the cloudiness was uniform.

The next step was to arrange five small tubes of uniform diameter, carefully cleaned, finally washed in distilled water and dried. Each one of the organisms, including a suspension prepared from the homologous organism, was tested in four steadily increasing dilutions. The amounts used and final dilutions reached are shown in Table V, No. 5 of the first column, representing a plain suspension in NaCl solution without serum, as a control:

TABLE V.

SERUM DILUTIONS.

Tube No.	Amount of Suspension	Serum (Undiluted)	Serum Diluted with 0.85% NaCl 1:10	Final Dilution
1.....	24 minims	1 minim	.....	1:25
2.....	16 "	.....	4 minims	1:50
3.....	18 "	.....	2 "	1:100
4.....	19 "	.....	1 "	1:200
5.....	25 "	.....	.....	.....

The tubes were placed in the incubator at 37° C. at 12 o'clock, and the first observations made at one o'clock. The second observations were made at two o'clock, the third at six and the final ones at eight o'clock the following morning, the tubes always being kept at 37° C. The testing of every set of organisms sent to the laboratory was accompanied by a control experiment with the homologous organism.

The results after 20 hours at 37° C. are given in Table VI. Agglutination usually began to appear in two hours. In six hours many of the reactions were complete, but especially in higher dilutions, reactions which were partial after six hours often became complete in twenty hours. The suspensions in NaCl solution never showed any agglutination.

TABLE VI.

AGGLUTINATION OF CULTURES BY IMMUNE RABBIT'S SERA.

CASE	CULTURE	IMMUNE RABBIT'S SERA											
		Shiga			Kruse			Flexner			"Y" of Hiss and Russell		
		+++	++	+	+++	++	+	+++	++	+	+++	++	+
11.....	6	...	...	...	...	...	...	...	...	...	...	...	...
15.....	1	...	...	...	50	...	100	...	...	...	25	50	100
17.....	1	...	...	...	...	25	50	100	200	...	25	50	100
19.....	1	...	...	...	...	...	...	25	50	100	...	...	...
19.....	30	50	...	100	...	25	50	25	50	200	...	...	...
27.....	1	...	...	...	No test			25	50	100	25	50	100
36.....	1	...	...	...	...	...	...	...	50	100	...	...	...
36 (2)..	1	...	...	...	50	100	200	...	...	...	...	25	50
37.....	1	25	50	100	...	...	...	...	...	...	25	50	100
39.....	1	50	100	200	...	...	...	100	...	200	...	...	...
43.....	5	...	...	...	...	...	...	...	...	...	...	...	...
54.....	3	...	...	...	...	...	...	25	50	100	50	100	200
55.....	11	50	100	200	25	50	100	50	100	200	50	100	200
56.....	1	100	200	...	...	...	...	...	25	50	50	100	200
76.....	3	...	...	...	...	25	50	...	...	...	25	50	100
78.....	1	50	100	200	...	...	...	50	100	200	...	...	...
82.....	1	100	200	...	25	50	100	50	100	200	25	50	100
83.....	1	25	50	100	25	50	100	...	...	...	50	100	...
86.....	1	...	25	50	...	...	...	...	...	...	50	...	100
93.....	1	...	...	...	...	...	...	...	...	...	25	50	100
93.....	3	...	...	...	...	...	...	...	...	...	...	...	...
96.....	1	...	...	...	...	25	50	...	...	...	...	...	...
101.....	1	50	100	200	...	...	...	...	...	...	...	...	...
101.....	5	50	100	200	...	...	...	...	...	...	...	...	...
102.....	1	...	25	50	50	100	200	...	...	50	...	25	50
102.....	2	...	...	...	...	...	...	100	200	...	...	...	...
102.....	6	25	50	100	...	...	...	100	200	...	...	...	...
103.....	7	...	...	...	...	25	50	...	...	...	...	25	50
109.....	4	25	50	100	...	...	...	...	...	...	...	...	...
110.....	1	25	50	100	...	...	...	50	100	200	50	100	200
A.....	1	...	...	...	...	...	...	...	...	...	...	...	...
B.....	1	...	...	...	...	...	...	100	200	...	...	...	...
B.....	4	...	...	...	...	...	...	100	200	...	50	100	200
Homologous cul.		100	200	...	50	100	200	100	...	200	100	200	...

The figures represent the dilutions at which agglutination took place after 20 hours at 37° C.

+++ = perfect clearing of supernated fluid, with the clumps of agglutinated bacilli settled to the bottom.

++ = a fairly complete result.

+ = a distinct clumping with a small amount of turbidity of the supernatant fluid.

A summary of this work shows that 29 of the 33 organisms examined gave positive results with one or more of the sera. Of these 29, nine agglutinated with a single serum, as follows: three

with Shiga serum, one with Kruse serum, four with Flexner serum, and one with "Y" serum.

Twelve agglutinated with two kinds of sera, as follows: three with Shiga and Flexner sera, two with Shiga and "Y" sera, four with Kruse and "Y" sera, three with Flexner and "Y" sera.

Six agglutinated with three kinds of sera: two with Shiga, Kruse and Flexner sera, one with Shiga, Kruse and "Y" sera, two with Shiga, Flexner and "Y" sera, one with Kruse, Flexner and "Y" sera.

Two organisms agglutinated with the four different sera employed.

III.

CLINICAL STUDY OF 97 CASES OF SUMMER DIARRHEA IN WHICH THE INTESTINAL CONTENTS WERE EXAMINED FOR DYSENTERY BACILLI.

MAY MICHAEL.

THIS report contains a synopsis of the clinical features of the cases of summer diarrhea studied bacteriologically by Dr. Weaver and his associates. The records were made at the Daily News Sanitarium by Dr. Coone, Dr. Vail, and the author. The character of this sanitarium, which is more of a temporary refuge than a place for permanent treatment and care, explains the incompleteness of some of these observations.

SYNOPSIS OF THE 97 CASES STUDIED.

Age and sex.—Of the 97 cases of summer diarrhea examined for dysentery bacilli 60 were males and 37 females. Sixteen cases were less than six months of age and of these four were severe, six moderately severe, six mild. Of 36 cases between six months and one year 14 were severe, 11 moderately severe, 11 mild. Of 40 cases between one and two years six were severe, 18 moderately severe, 16 mild, and of four cases over two years one was moderately severe, three mild. Consequently the majority of the cases occurred between one and two years. It seemed that cases between six months and one year were most severe.

Method of feeding.—Only nine of the children were breast fed at the time of the attack; 16 others were partly breast-fed. Of these 16, two received, in addition to the breast, condensed milk, 12 mixed diet, one laboratory milk, one Mellin's food. Of the 66 artificially fed children 43 had

general diet, 10 milk or modified milk, eight condensed milk, two Eskay's food, one oatmeal gruel. Hence the largest number of cases occurred in artificially fed infants.

Predisposing causes.—In only 24 cases did the attack seem to depend upon special conditions: in three cases it followed weaning, in two change of diet, in 19 the feeding had been manifestly improper.

Principal symptoms.—In 45 cases the stools contained mucus and blood: in 28 only mucus; in 23 neither mucus nor blood; in one case there is no record.

The number of stools varied from 2 to thirty in 24 hours. In about half of the cases there were from two to ten stools in 24 hours.

Tenesmus occurred in 23, vomiting in 52, persistent vomiting in seven.

Complications.—Eczema in two, furunculosis in three, bronchitis in 16, pneumonia in two, stomatitis in seven, prolapse of rectum in five, convulsions in three. In 28 cases there was evidence of rickets; all except eight were or had been breast-fed; in 10 of these cases the attack was severe, in 12 moderately severe, in six mild.

Termination.—Three died, 54 improved, 11 remained stationary, and of 29 there was no final record left at the sanitarium.

These 97 cases may fairly well be divided into two groups: (1) Cases of gastro-intestinal catarrh, or fermental diarrhea, in which the toxic symptoms predominated; (2) Cases of follicular enteritis, ileocolitis or colitis, in which the inflammatory symptoms predominated.

Cases of the first group were characterized by sudden onset, loss of appetite, vomiting, and diarrhea. The fever ranged from 99° to 101° in mild cases, and rose to 104°–105° in the severe ones. The stools numbered from 6–10 a day, were yellow or green and slimy, thin and watery, or brown and offensive: they often contained undigested food and mucus. The child was restless, with dry mouth and coated tongue. Sometimes, if the toxemia was great, convulsions occurred at the onset and the disease ran a severe course with continued high fever and marked nervous symptoms.

Complications were not common, but at times eczema, furunculosis, stomatitis, whooping-cough, bronchitis, and pneumonia occurred. Recovery within five to six days was the general termination, although the disease occasionally became chronic and lasted for weeks.

In the second group the inflammatory symptoms were more marked. The onset was acute, the attacks being ushered in by diarrhea, often accompanied by vomiting; the stools, at first fecal,

soon consisted almost entirely of mucus or mucus and blood, this last element varying from a few flecks to a large amount. The temperature was, as a rule, but slightly elevated, 100–101°, although at times normal or subnormal, and sometimes at the beginning very high. Tenesmus was usually present and often associated with prolapse of the rectum. Abdominal pain was frequent and quite intense just before stool. These cases ran a variable course from one to six weeks, but recovery was the rule.

SYNOPSIS OF 24 CASES OF SUMMER DIARRHEA FROM THE STOOLS OF WHICH DYSENTERY BACILLI WERE ISOLATED.

Age.— Under six months, one; between six and twelve months, two; between one and two years, 15.

Method of Feeding.— Breast-fed, three: partly breast-fed, three: artificially fed, 12 (eight, general diet; two, cow's milk; two, condensed milk); no record, six.

Principal Symptoms.— In 22 cases the stools contained mucus and blood, in two only mucus. Tenesmus occurred in 11 cases and vomiting in 13; rectal prolapse in two; bronchitis in four.

Termination.— Recovered or improved, ten; unimproved, five: died, two; no final record, seven.

Hence cases associated with dysentery bacilli in the stools presented more the clinical picture of ileocolitis.

The question whether infection with B. dysenteriæ causes a clinical entity has been discussed by a number of writers. Dorothy Reed[1] has studied a series of cases in which B. dysenteriæ was isolated from the stools and has drawn the following conclusions: "blood and mucus or much mucus in the stools, where there is no occasion for such an appearance, as intussusception, extreme purgation, etc., indicates infection with the B. dysenteriæ; even a little mucus points to such an infection if such stools have existed for a long time. A certain number of cases of infantile diarrhea present a symptom complex comparable to adult dysentery and are caused by the B. dysenteriæ."

Knox[2] says that among children, diarrheas due to B. dysenteriæ cannot be differentiated from the ordinary summer diarrheas.

Hastings[3] has found the Shiga bacillus in cases diagnosed clinically as follows: ileocolitis (25), cholera infantum (one), ente-

[1] *Studies from Rockefeller Institute for Medical Research*, 1904, 2, p. 175.

[2] *Jour. Am. Med. Assn.*, 1903, 41, p. 173.

[3] *Ibid.*, 1904, 42, p. 1121.

rocolitis (two), fermental diarrhea (one), gastro-enteritis (two), enteritis (four).

La Fetra and Howland[1] believe that all types of diarrheal disease as characterized by their clinical symptoms are to be found among these cases (62 cases in which the Shiga bacillus was found); some of these were examples of severe and some of mild ileocolitis; others could be classed only as the mildest form of intestinal indigestion.

Holt[2] drawing his conclusion from all the cases studied by the Rockefeller Institute says: "Infection with the dysentery bacillus is associated with almost every sort of intestinal disturbance accompanied by diarrhea, except the severe acute intestinal intoxication, cholera infantum. The B. dysenteriæ is associated with inflammatory forms of diarrhea of all degrees of severity, mildest, most severe, acute, protracted, subacute; occurring both as a primary disease and a secondary disease, often occurring in institutions as a terminal infection in infants suffering from marasmus."

Rotch,[3] from the study of a series of cases, concludes: (1) That there are no specific symptoms besides the presence of B. dysenteriæ in the discharges to determine that B. dysenteriæ is the cause of an especial case of ileocolitis; (2) that B. dysenteriæ may cause the clinical type known as fermental diarrhea or as ileocolitis.

Koplik,[4] in discussing the question, says the bacilliary diarrheas occur for the most part in older children, although they may occur in very young children and have been found in institutions or in certain localities. They are a distinctly limited class of diarrheas and do not include all the forms which are gradually being controlled by modern methods of infant feeding.

Duval and Bassett[5] state that summer diarrheas are caused by intestinal infection with B. dysenteriæ Shiga, and are therefore etiologically identical with acute bacilliary dysentery of adults. These cases from which the dysentery bacillus was isolated include

[1] *Studies from Rockefeller Institute of Medical Research*, 1904, 11, p. 137.

[2] *Ibid.*, p. 185. [3] *N. Y. State Jour. of Med.*, 1904, 4, p. 173.

[4] *Archives of Pediatrics*, 1903, 20, p. 808.

[5] *Studies from Rockefeller Institute for Medical Research*, 1904, 2, p. 7.

examples of so-called dyspeptic diarrhea, enterocolitis, malnutrition, and marasmus with superimposed infection.

Clinically the 24 of our 97 cases in which dysentery bacilli were found did not differ from the cases of ileocolitis in which the dysentery bacilli were not found.

The success which has been attained in Japan with the specific serum treatment of dysentery in adults encouraged its use in infantile dysentery. According to Shiga the mortality in adult dysentery by its use was reduced to one-third below that given by the symptomatic treatment. On the whole, however, the results in infantile dysentery are disappointing. Only 12 of the 87 cases reported by the Rockefeller Institute showed any improvement attributable to its use. Holt[1] says the conditions for success in the use of the serum are, first that it must be used early before serious lesions have developed, and second that it must be used in repeated doses (10 c.c. daily in moderate cases, repeated two or three times daily for several days, in severe ones). He believes that the promising cases are the sharp acute attacks with symptoms of severe infection, where the real problem is to combat the infection, not to maintain the nutrition; also that inasmuch as two days are required for bacteriological diagnosis, if used at all, the serum must be injected on a clinical diagnosis. The variety of serum usually used is that obtained by immunizing animals with both the "Harris-Flexner" and the "Shiga" types of bacilli. The serum was used in only two of our series of cases; neither showed marked improvement from its use. As Holt says, a more extended trial upon more carefully selected cases is necessary before definite statement can be made as to the value of antidysenteric serum.

CONCLUSIONS.

I. Dysentery bacilli were not found in all cases characterized by mucus and bloody stools.

II. The group of cases in which dysentery bacilli were isolated from the stools presented the clinical picture of ileocolitis.

III. Cases of ileocolitis in which dysentery bacilli were isolated from the stools did not differ clinically from cases of ileocolitis in which these bacilli were not found.

[1] *Loc. cit.*

IDENTIFICATION OF ALCOHOL-SOLUBLE HEMOLYSINS IN BLOOD SERUM.*

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In a preliminary communication[1] it has been reported that ethyl-alcohol extracts from blood serum of various animals contain a substance or substances which lake red blood corpuscles held in suspension in 0.8 per cent. NaCl solution.

These hemolytically active extracts may be obtained from fresh serum, from dialysed serum, indeed from fresh serum which has been heated to any temperature up to 100° C. The lysin is therefore chemically quite stable; it is not generated by decomposition processes which may occur in blood serum, nor is it a product of the enzymatic process which Hahn[2] has recently shown occurs in blood of normal animals after withdrawal from the animal body; and its formation does not depend—wholly at least—(as may be inferred more fully from what will be presented farther on) on the action of the lipase of blood serum described by Hanriot.[3]

I have usually prepared it from serum, which, after being mixed with a few drops of chloroform, has been dried slowly in an incubator. The bone-dry serum is pulverized, then brought into a Soxhlet extraction apparatus and digested for several hours with petroleum ether. After this treatment the serum is digested with ethyl alcohol, preferably 60–75 per cent. Absolute alcohol removes some of the lysin also, but not to the extent that alcohol containing water does, which obviously penetrates the vitreous dried serum better than the dry absolute alcohol. The digestion fluid is filtered into wide, flat dishes and allowed to evaporate slowly in an incubator to complete dryness. The presence of

* Received for publication December 1, 1904.

[1] *Trans. of the Chicago Path. Soc.*, 1904, 6, p. 128.

[2] *Münch. med. Wchnschr.*, No. 16, 1904, 51, p. 689.

[3] *Comp. rend. heb. des séances de l'académie des sciences*, 1896, 123, p. 753; 1897, 124, pp. 235, 778.

the hemolysin in such extracts does not depend entirely—if at all—on any alteration which may occur in the serum (lipase) or in the extract (influence of light) during the drying in the incubator, because mixing fresh blood serum with two parts of 96 per cent. alcohol, drawing off the supernatant fluid after centrifuging the mixture, and evaporating it in a vacuum removed from sunlight also yields a hemolytic extract. Such extracts are, however, not as active hemolytically as those obtained from dried serum and dried in an incubator. It is possible, therefore, that even the slow dryings in the incubator are accompanied by some changes in the serum and in the composition of the extract which intensifies its hemolytic properties; but the effect of drying in the incubator is by no means essential to the formation of the lytic substance.

The extracts after being freed completely from alcohol are mixed with sterilized 0.8 per cent. NaCl solution of a volume equal to the volume of serum used at the outset. This mixture furnishes a permanent emulsion which is neutral to litmus.

The emulsions lake red blood corpuscles of any animal which has been tested, viz.: sheep, goat, rabbit, ox, hog, guinea pig, dog, and man. Usually about 0.2 c.c. of an emulsion suffice to cause complete laking of one c.c. of a five per cent. suspension of washed corpuscles. The degree of lytic activity of the emulsions varies, however. The minimum amount of an emulsion observed to cause any appreciable hemolysis is 0.02 c.c. Lysis occurs readily at ordinary room temperature, and even at 0° C. The same emulsion acting on corpuscles of different species of animals produces different degrees of hemolysis, sheep corpuscles being apparently the most resistant, and dog corpuscles the most susceptible.

Heating the emulsions, even to 100° C. for three hours, causes no apparent alteration in their hemolytic properties.

The lysin is not in the menstruum, but rather in the suspended matter of the emulsions. For if an emulsion be passed through a Chamberland porcelain filter, the yellow filtrate, if perfectly clear, usually has no lytic action on erythrocytes. If, on the other hand, the residue be dissolved from the filter with alcohol,

the clear solution which is obtained leaves, on being evaporated, a residue which, re-emulsified in salt solution, hemolyses quite as actively as the original emulsion.

The lytic action of these emulsions on the corpuscles of any species of animal is inhibited by the blood serum from the same species. Heated sera from other species may also have antilytic properties, but not of the same degree toward the corpuscles of all species. For instance, laking of dog corpuscles by an active emulsion was not prevented by the same amounts of heated sheep or dog serum as suspended lysis of sheep or guinea pig corpuscles. The watery fluid which exudes from the coagula of boiled serum also has antilytic properties; the same is true of serum which has been dialyzed for forty-two hours and then mixed with an equal volume of 1.6 per cent. NaCl solution.

As for the origin of this lysin: it is not furnished by the white corpuscles, as might be suggested. Alcohol extracts made from white blood corpuscles, obtained from the pleural cavity of a rabbit after injection of aleuronat did not contain it. It was present to a very slight extent in an extract from pleural fluid and also in one from ascites fluid.

The hemolytic properties of these extracts, in so far as similar tests have been made of them, correspond quite closely to those of the organ extracts which Korschun and Morgenroth[1] made by digestions of the organs of animals in physiological salt solution. The probability of the identity of at least one of the active components of the extracts here described and that in their extracts is very strong.

The known constituents of blood serum which are extractable by alcohol but which are not soluble in water and which therefore may be sought in the suspended matter of these emulsions are: fats, cholesterin, cholesterin-esters of fatty acids, lecithin, jecorin fatty acids, and insoluble soaps. Blood serum is said to contain some soluble soap which would of course be extracted by alcohol. The soluble soaps have an intense lytic action on erythrocytes. Since, however, the filtrates obtained by passing the emulsions through the Chamberland filter usually do not lake, it may be

[1] *Berl. klin. Wchnschr.*, 1902, 39, p. 870.

taken for granted that soluble soap is not usually present in the emulsions in sufficient quantity to be a factor in their hemolytic action. The method of extraction used in making the emulsions here described by no means exhausts all the matter extractable by alcohol from blood serum. The amount of extract yielded by boiling fresh serum four or five times with alcohol in a flask fitted with a return condenser is of course much larger than the amounts obtained by the mild procedure actually used. The cold extractions and drying at low temperatures were adhered to in order to obtain the extractable substances more nearly in the state in which they are present in the normal serum. It is known that constituents of blood serum — as jecorin for instance — would certainly be decomposed by a hot extraction by alcohol.

An emulsion of the extract obtained from pulverized dried serum by digesting it for twelve hours with petroleum ether in a Soxhlet extraction apparatus did not lake red corpuscles. This extract would contain fats, probably cholesterin, cholesterin-esters, lecithin, and possibly jecorin. Extracts from dried serum obtained with ethyl ether or with chloroform have hemolytic properties. Such extracts may contain in addition to the substances extracted by petroleum ether fatty acids and insoluble soaps.

To ascertain exactly what the lytic agent is in the alcohol extracts the following procedure was adopted: Pulverized dried serum was digested for twelve hours with petroleum ether in a Soxhlet apparatus. The dried serum was then freed from petroleum ether and treated several times in the course of three days with 60–90% alcohol. The digestion fluid was filtered into a wide, flat dish and allowed to evaporate in an incubator at first, then for two days in a calcium-chloride chamber in the incubator. The now thoroughly dried extract was treated several times with absolute alcohol, the dissolved portion filtered into a dish and again allowed to dry in an incubator and afterwards in the calcium-chloride chamber. The residue of the extract insoluble in absolute alcohol was freed from alcohol and emulsified in salt solution; it was found to have no hemolytic power.

The substance soluble in absolute alcohol, after thorough drying was brought on filter paper into a Soxhlet apparatus and digested at the lowest possible temperature with perfectly water-free petroleum ether. The substance thus dissolved out by petroleum ether, after expulsion of the petroleum ether, was tested for fat and cholesterin with positive results, for lecithin and jecorin with negative results. The substance was then emulsified and found to be non-hemolytic. Thus it was shown that neither fats, cholesterin nor cholesterin-esters are active as hemolytic agents, and since lecithin and jecorin cannot be shown to be present in the extracts they do not come into question as factors in the hemolytic action of the emulsions obtained from serum with weak alcohol.

The portion of the absolute alcohol extract insoluble in petroleum ether was mixed with water, brought into a separating funnel and treated in different cases with chloroform and with ethyl ether. With chloroform a substance was withdrawn from the aqueous mixture, which being dried and emulsified was strongly hemolytic. The aqueous mixture left after the exhaustion with chloroform was evaporated and the resultant residue emulsified and found to be non-hemolytic. With ethyl ether an extract was obtained from the substance left undissolved by petroleum ether which hemolyses and the emulsion of the dried residue was hemolytic also.

Now the substances removable from the aqueous emulsion of the material insoluble in petroleum ether by both chloroform and ethyl ether are the fatty acids. They are undoubtedly the chief factors in the hemolysis caused by these emulsions. Oleic, stearic, and palmitic acids may be emulsified in small quantities in 0.8% NaCl solutions and such emulsions lake red blood corpuscles with surprising facility. The amount of oleic acid held in suspension in 10 c.c. of salt solution is not ordinarily strong enough to neutralize 1.0 c.c. of $\frac{N}{50}$ NaOH solution. Yet one c.c of such an emulsion dissolves the erythrocytes in one c.c. of suspended corpuscles almost immediately and 0.1 c.c. dissolves them almost completely after a few hours. Stearic acid is less active, and palmitic acid is the least active of the three. The

unanalyzed active emulsions derived from blood serum, titrated with $\frac{N}{50}$ NaOH solution, using phenolphthalein as indicator, always show the presence of free fatty acid. The amounts of $\frac{N}{50}$ NaOH solution required to neutralize 20 c.c. of an emulsion being usually 0.8 c.c. to 1.5 c.c. The presence of even that much free fatty acid in an emulsion would, according to tests with prepared emulsions of pure fatty acids in physiological salt solution, almost wholly account for its hemolytic properties. There may be some doubt, however, if even such small amounts of free fatty acids are to be found in blood serum with its normal alkalinity. It is a question if they are not the products of the action of lipase, which by the method usually followed of making the extracts is not to be excluded; furthermore the decomposing effects which light and air are known to have on some fatty compounds might be a source.

Be that as it may, the treatment of an emulsion with ethyl ether which should take up all the fatty acids present does not deprive it wholly of its hemolytic properties. Treatment of dry extracts with chloroform sometimes leaves something in the insoluble residue which in emulsion is hemolytic. Now the substances left by ethyl ether in an emulsion, which one might suspect, would be the soaps. For reasons already set forth it cannot be supposed that any considerable amount of soluble soap is present in the extracts. There remain, then, the insoluble soaps to be considered.

A preparation of magnesium soap made either from ordinary soap or from a solution of pure sodium oleate can be emulsified and held in fine suspension in NaCl solution. Such emulsions, which are perfectly neutral, even to phenolphthalein, have the property of laking red blood corpuscles on being mixed with them. This laking is inhibited by a small quantity of serum; so it cannot be said that the antilytic properties of serum toward these emulsions is to be ascribed to a neutralizing effect which the alkali of the serum may have on free fatty acids. Moreover, this would form soluble soap, which we know is a laking agent. After standing several days the suspended matter of a magnesium soap emulsion gathers together and leaves a clear men-

struum, which on being titrated with silver nitrate solution is shown still to contain just as much chloride of sodium per cubic centimeter as the NaCl solution used. It cannot be said, therefore, that the lytic properties of an emulsion of magnesium soap in NaCl solution are to be ascribed to a hypotonicity of the salt solution brought about by the suspended matter attaching some of the NaCl and thus withdrawing it from the solution.

Calcium soaps prepared in the same way are not so emulsifiable and do not have hemolytic properties.

There is no mention by physiologists that the magnesium and calcium of blood serum are there in combination with fatty acids. Yet, knowing that when blood serum is incinerated, more metallic oxides are found in the ash than can be accounted for by organic acids known to be present, the excess of bases would be explained by the fact that they are in combination with some unknown inorganic acids. Magnesium soap is often found in the intestines, and it is known that by the aid of bile it can readily pass through the intestinal wall, but its fate has never been further traced. The mystery presented by blood serum containing an incompatible mixture such as magnesium and phosphoric acid, calcium and carbonic acid, supposedly all in a solution together, has been explained thus: Perhaps the albumins of a serum have the faculty of holding these substances in solution as water cannot. But the possibility of a combination of calcium and magnesium with fatty acids and held in emulsion in the blood serum removes the mystery. Now, if magnesium soaps are present in the blood serum, they may be included in the substances of the alcohol extracts of blood serum which are hemolytic. A faultless demonstration of the presence of magnesium soaps in the extracts cannot be made so easily, however, on account of the presence of the free fatty acids. A perfect isolation of the fatty acids from the magnesium soaps is very difficult, since all utilizable solvents of the former either partially dissolve or emulsify magnesium soaps. This difficulty is much heightened by the fact that the amounts of these substances in our emulsions are at best very minute. Numerous incinerations of active emulsions have always shown the presence of magnesium and sometimes of

calcium in the ash—in the ash derived from substances exhausted with ethyl ether or chloroform from absolute alcohol extracts, as well as in the residues not taken up by these solvents. There are, one may assert at least, no other known compounds of magnesium in blood serum which are soluble successively in absolute alcohol and chloroform or in absolute alcohol and ether. Hence one is warranted in making the assertion that magnesium soaps are present in blood serum and, that being so, they are factors in the hemolytic action of extracts of blood serum.

This would naturally lead to the inquiry as to whether magnesium soaps, which are evidently present in blood serum in larger quantities than the alcohol extractions from dried serum furnish, may not be concerned in some way with the action of the complex hemolysins of the blood; whether they, for instance, may not be connected in some way with the more stable component of the hemolysins, the amboceptor. To gain a possible clue an endeavor was made, (1) to ascertain if the amounts of calcium and magnesium in the blood serum of an immunized animal showed increase over that of a normal animal; (2) to ascertain if heated immune serum, after being mixed with suitable washed corpuscles, was deprived of appreciable amounts of magnesium or calcium, since the corpuscles absorb the amboceptor. With these considerations in mind 80 c.c. of blood serum from a goat which had been injected periodically with sheep's corpuscles, so that just before the analysis 0.005 c.c. laked completely one c.c. of a five per cent. suspension of washed sheep corpuscles, was heated at 62° C. for one-half hour. The serum was now no longer hemolytic. It was then divided into two equal parts. One part was evaporated to dryness in a large porcelain crucible. The other portion was treated with the washed corpuscles from 100 c.c. of sheep's blood at 36° C. for two hours, then centrifuged and separated from the corpuscles. The corpuscles were then mixed with 0.8 % NaCl solution twice, centrifuged, and separated from the supernatant fluid which was added to the serum. The serum and the corpuscles were then evaporated to dryness separately in porcelain crucibles. Washed corpuscles from 100 c.c. of the same sheep's blood without being treated with goat serum were also

evaporated to dryness in a crucible. Finally 40 c.c. of blood serum from a normal goat was evaporated to dryness. After thorough drying of the contents of these five crucibles they were carefully incinerated.

The ashes resulting from the incineration were dissolved in hydrochloric acid and the resulting solutions analyzed for their calcium and magnesium content. This was done by rendering the solutions ammoniacal, then slightly acid with acetic acid. Any iron present was eliminated as phosphate by adding a small quantity of disodiumphosphate (to insure a sufficiency of phosphoric acid) and filtering. In the filtrate acidified by acetic acid the calcium was precipitated by ammonium oxalate, filtered off and weighed as oxide. The filtrate was made ammonical and after standing the magnesium precipitate was collected by filtration and weighed as pyrophosphate. The results of these determinations of the calcium and magnesium in the sera and corpuscles were as follows:

1. 40 c.c. normal goat serum contained CaO 0.0054 gr., MgO 0.00082 gr.
2. 40 c.c. immune " " " " 0.0064 gr., " 0.00243 gr.
3. 40 c.c. immune goat serum, treated with washed sheep's corpuscles, centrifuged and drawn off clear " " 0.00625 gr., " 0.00225 gr.
4. Corpuscles from 100 c.c. blood which had been exposed to heated immunized goat serm " " 0.00307 gr., " 0.00182 gr.
5. Washed corpuscles from 100 c.c. sheep's blood " " 0.00255 gr., " 0.00212 gr.

Control determinations of the calcium and magnesium in (4) and (5) by the more exact method of Chizynski gave approximately the same results as above. The assay of such minute amounts of these substances is necessarily attended by proportionally large errors. However, the results obtained for the amounts of CaO and MgO contained in immunized serum before and after mixture with corpuscles and that contained in sensitized and non-sensitized corpuscles correspond nearly enough to make it quite certain that there is no interchange of these substances of the kind suspected. Of interest is the fact that the analyses

show a smaller amount of MgO in normal serum than in immunized serum. Whether this relation holds good between all normal and immunized animals as well as between the two from which the sera used in the above determinations were obtained would be important to ascertain. This will be looked into further as soon as other immunized animals are available.

Although a relation of magnesium soaps to the complex hemolysins of the blood has not been established by these experiments, their existence in blood serum and their hemolytic properties are quite a certainty. A confirmation of the observation of Hahn that by the action of an enzyme fat is formed in normal blood but not in immunized blood *in vitro* would be a positive indication of some relation of the hemolysin either to fats, derivatives of fats, their progenitors or the fat-forming agent. As already stated, the fatty acids in the alcohol extracts from blood are probably products of the manipulation, and though one might even suspect the magnesium soap to be of the same origin, the possibility of establishing a relation between fat substances in blood and complex hemolysins makes this inquiry one which merits further pursuit.

In conclusion I wish to thank Dr. L. Hektoen, who suggested this work, for many valuable hints and constant courtesy.

PROTECTIVE INOCULATION AGAINST ASIATIC CHOLERA.*

AN EXPERIMENTAL STUDY.

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THE epidemic of Asiatic cholera which has recently passed through the Philippine Islands has brought forcibly before us the particular difficulties encountered in combating and controlling a disease of this nature in a tropical country and among a partly uneducated people. Moreover, its history has demonstrated the impossibility of eradicating or even of satisfactorily controlling the malady in this city by ordinary hygienic methods, that is, by those measures directed solely towards the purification of the food and water supply of the infected districts. During the period at which the number of infected individuals was the greatest, it was shown by studies made in this laboratory that, in Manila at least, the disease was not usually transmitted directly by water,[2] but more often by food infection. Under the conditions existing here, an individual suffering with cholera or convalescent from it might frequently infect the food of healthy persons. Although the Board of Health took every precaution practicable with regard to the furnishing of uninfected water, the prohibition of the sale of many fruits and other raw food material, and the isolation and treatment of the sick, nevertheless the epidemic spread, continued for nearly two years, and caused the death of 3,866 people in the city of Manila alone. Hence, it was evident,

* Received for publication September 15, 1904.

[1] The experimental work which forms the basis of this article was for the greater part performed during the spring of 1903 in the "Institut für Infektionskrankheiten," Berlin (Professor R. Koch, Director, Department of Professor A. Wassermann). I wish to express here my very grateful thanks to Professor A. Wassermann, under whose direction the research was first undertaken, for many suggestions and courtesies during the course of my studies.

[2] In other portions of the Archipelago the disease was certainly conveyed and spread by the water supply.

at least with a population of this character, that since it was not possible to prevent many individuals from coming into contact with and even ingesting the cholera organism, it would be advisable to immunize them artificially and to protect them by vaccination against the disease.

HISTORICAL.

The methods which have been employed for human protective inoculation against cholera are not numerous. Ferran, in an epidemic which raged in Spain, was the first to introduce the vaccination of human beings against the disease. Several government commissions were appointed to investigate Ferran's method, but their opinions as to its merits were for the most part unfavorable. Gamaleia then suggested the use of sterilized virulent cultures for the injection, but Haffkine returned to the use of living cultures, making a first inoculation from a standard attenuated culture and using a virulent culture for a later treatment. Afterwards, following Tamancheff, Haffkine recommended the addition of a 0.5% solution of carbolic acid to the culture. Kolle maintainted that a single injection of living or dead cholera vibrios gave as good an immunity as did the repetition of the dose. Patients whose serum normally had a value of 0.75-0.6, showed ten days after inoculation a serum value of .003 grms., *i. e.*, 0.003 grms., protected guinea pigs against ten times the fatal dose of cholera. Kolle recommended the following method: a well-grown agar culture containing about 20 mg. of growth is emulsified in 10 c.c. of physiological salt solution, and sterilized for a few minutes at 50° C.; 0.5% phenol may be added. In vaccinating, one c.c., equal to two mg. of the culture, is injected subcutaneously. Larger amounts were occasionally employed by Kolle.

Several investigators, Klemperer, Ketscher, and Sawtchenko and Sabotolny, have endeavored to produce immunity against cholera by feeding experiments, but this method, being tedious and uncertain, has obtained no practical acceptance. Various vaccines have also been prepared, some of them chemically, by Brieger and Wassermann, Federoff, Wassermann, Klebs, Rosminski: Issaeff attempted to increase natural resistance by inducing phagocytosis; Buchner and Hahn extracted by pressure a so-called cholera plasmin which was used for inoculation; Emmerich and Loew asserted the efficiency of "pyocyanase" against the cholera vibrio; and finally Besredka used for inoculation organisms which had been saturated with immune serum.

Passive immunization against Asiatic cholera by the use of antitoxic or bactericidal sera need not be discussed here, as it is obvious, because of the brief immunity which such sera confer, that their value as a practical prophylactic is very limited.

From this review it is evident that as yet no satisfactory form of protective inoculation against cholera has been established. Usually those prophylactics which give rise to the necessary

immunity produce too severe a local reaction; while no other, causing but a mild local reaction, has proved sufficiently protective. Of the methods employed, those of Haffkine and of Kolle promise the best results, but a few preliminary trials with Haffkine's method showed its impracticability for these Islands; first because, on account of the severe local and general reaction, the natives would not voluntarily submit to it; secondly, because on account of this violent reaction and of the unsettled condition of the country, it was inadvisable to make such vaccination compulsory; lastly, because, while this inoculation gives rise to a bactericidal and agglutinative serum in the inoculated, the antitoxic value of such serum is probably very slight.

On account of the great importance of this question, an experimental study was undertaken with the object of obtaining some practicable and efficacious form of inoculation against the disease.

DESCRIPTION OF THE CULTURES.

In my search for practical vaccine, I first studied the local reaction and other toxic effects produced in animals after the injection both of a very virulent cholera culture and of one which, through long cultivation upon artificial media, had lost most of its virulence. The effects of the killed as well as of the living organisms were also studied with each culture. These two races, for the sake of brevity, will be referred to in this article as "Virulent" and "Avirulent."

The avirulent organism was obtained through the kindness of Professor Wassermann. It had been isolated by R. Pfeiffer in the epidemic of cholera which occurred in Hamburg in 1894, and preserved for nine years on artificial media, with passage through animals from time to time. During the preceding year, however, the strain employed in these experiments had been grown only on artificial media, and not inoculated into animals. Its growth on all culture media was typical for Spirillum choleræ asiaticæ, including the production of nitroso-indol in peptone solution. In its morphological characteristics it was not typical, in that short commas were seldom observed, but instead rather long threadlike forms; also its motility was lost to a great extent, though not entirely. However, it was agglutinated by a standard cholera immune serum in even higher dilutions than was another genuine cholera strain; and there is no doubt of its representing a true though attenuated type of Spirillum choleræ asiaticæ.

The virulent organism was isolated by Professor Kolle in Jaffa during the recent epidemic of cholera there. It reacted typically in all media, and its morphology and motility were also characteristic of the genuine cholera organism. It was agglutinated in high dilutions by the cholera immune serum above mentioned, though in less degree than the avirulent strain.

These cultures were accurately standardized, and the minimal lethal dose for guinea pigs of 250 grams' weight determined. After numerous passages of "Virulent" through animals, a lethal dose of 0.1 of a standard loop[1] (two mg.) of a 20-hour agar culture was reached. Such a dose of "Virulent," when suspended in one c.c. of a 0.85% NaCl solution and injected intraperitoneally into a guinea pig of 250 grams' weight, regularly caused death within 24 hours. With "Avirulent," on the other hand, 1.5 standard loop of a 20-hour agar culture, injected intraperitoneally, was required to produce death within the same time in such an animal. The former culture, therefore, may be said to possess 15 times the virulence of the latter. Throughout the course of the work this relationship between the two organisms was carefully preserved and continually tested by inoculation, a precaution especially necessary because of the rapid change in virulence of the cholera spirilla when grown on laboratory media.

METHOD.

The reactions for agglutination were performed in the test-tube. One loop of the living organisms was thoroughly emulsified with one c.c. of an 0.85% NaCl solution. The diluted serum, suspended in one c.c. of a similar salt solution, was then added, the tube well shaken, and the mixture allowed to stand for two hours at 37° C. In a complete agglutination the liquid overlying the precipitated bacteria is entirely clear; in a weak reaction, although there is a distinct agglutination, with a precipitation visible to the naked eye, the supernatant fluid remains more or less cloudy.

The bactericidal reactions were tested in the abdominal cavity of guinea pigs according to the method of R. Pfeiffer, a syringe with a blunt-pointed needle being employed. Care was taken to avoid injury to the intestine. The dilutions of the serum were made in 0.85% NaCl solution. One cubic centimeter of the diluted serum was then added to one c.c. of broth containing two loops of "Virulent" in suspension, after which one c.c. of the resulting mixture was injected into the peritoneal cavity of a guinea pig of 250 grams' weight (or a little less), the animal thus receiving 10 times the fatal dose of the living organisms. A fresh guinea pig was of course used for each reaction. The experiment was controlled by microscopic examination of a drop of serum from the abdominal cavity, obtained by means of a capillary tube; this was taken immediately and again 20 minutes after the inoculation. Control animals were also inoculated with 10 times the fatal dose of "Virulent," but without serum. The result to the animal after 24 hours, whether it was then living or dead, was regarded as the final test, though the condition of the organisms in the abdominal cavity after 20 minutes was always carefully noted.

It soon became evident that the local reaction upon the tissues after a subcutaneous injection of "Avirulent" was much less than after one with the virulent cholera spirillum. I therefore decided to determine the character of the serum which could be produced with this avirulent organism and to compare it with that produced by the virulent germ.

[1] This standard loop was employed throughout the work.

Rabbit series No. I.—A series of six rabbits of an average weight of 1,500 grams were inoculated intravenously, three each with one-half loop of "Virulent" and three each with one-half loop of "Avirulent," the organisms in every instance being suspended in one c.c. of broth. After eight days the rabbits were all killed by bleeding, and the agglutinative and bactericidal value of the serum determined in each case. Rabbits inoculated with the virulent culture furnished better serum than those inoculated with the avirulent, but the value in both agglutinative and bactericidal properties of the serum from the animals treated with the former was in no case more than two and one-half times that of the serum furnished by animals treated with the latter strain.[1]

These results were somewhat at variance with those of Haffkine, and still more so with those of F. Pfeiffer and Friedberger, who used dead cholera spirilla of different degrees of virulence. It was therefore decided to repeat the experiment, the virulence of the cultures being determined as before on the day of inoculation. The result was practically the same, the serum showing at the end of eight days that the virulent strain had in but one case given a serum of more than two and a quarter times the bactericidal value of that produced by the avirulent. In this one case the avirulent serum was between one-fourth and one-fifth as strong as the virulent serum. From the results of those experiments with intravenous injection of *living* organisms into rabbits we might apparently assume that the immunity produced is not directly proportional to the virulence of the inoculated organism.[2]

In the experiments performed with subcutaneous injection of the avirulent organism, both living and dead, the local reaction, as already mentioned, was much milder than with the use of the virulent germ; but there was always considerable local inflammation about the point of inoculation. Hence it appeared desirable to do away, if possible, with the bacterial cells.

In this connection it is necessary to recall that a satisfactory protective against Asiatic cholera must probably contain substances which give rise to antitoxic as well as bactericidal bodies in the blood sera of the inoculated. A consideration of both these substances was thus necessary; and the problem of their extraction from the bodies of the bacteria was next investigated.

[1] Tables giving the details of these and other experiments appear in full in Bull. No. 16, 1904, of the Bureau of Government Laboratories, Manila, P. I.

[2] These questions of the relation between immunity produced and virulence of culture used will be discussed in more detail in a later paper.

The toxin.—In epidemic cholera the symptoms often develop very rapidly after they have once begun, so that, usually within a few hours after their appearance, either death or a severe state of collapse has supervened. The cholera process cannot be satisfactorily explained except as the effect of an organic poison, and it therefore appears likely that in a very short space of time a considerable amount of toxic material is manufactured and liberated. Further, cultures made from the intestinal material during the period of most acute symptoms and compared with those taken 48 hours or more after the entrance of the stage of collapse show that in the latter condition there is usually a remarkable reduction in number of cholera spirilla and apparently a great increase in the number of other intestinal bacteria. If this clinical experience be compared with the observations made in the laboratory, namely, that in agar cultures of cholera spirilla the maximum growth at 37° C. is obtained in from 12 to 20 hours, and that after this time a rapid death of the spirilla takes place,[1] it may be inferred that possibly the stage of the most violent symptoms in human cholera corresponds with the period during which the rapid dying off of the spirilla occurs. It might also be possible that at the time of death of the spirilla the largest amount of toxic substance is set free.

The isolation of this specific substance, the existence of which was first argued by Koch, has been often attempted, by Hueppe among the first, and by many other investigators; but today it would appear that none of these authors was dealing with the specific cholera toxin alone. Pfeiffer failed to demonstrate any soluble toxin in young broth cultures, and only a ptomaine-like body in old broth cultures. He was able to show, however, a powerful toxic substance in killed fresh agar cultures. His work has been confirmed by others, among them Kolle and Wassermann, who show that the true cholera toxin exists as a constituent element of the bacterial cell. This poison becomes soluble only by the disintegration of the bodies of the bacteria, and the fluid culture is very unstable and soon destroyed. It is true, Metchnikoff and Roux profess to have demonstrated a soluble cholera toxin by its action through a collodion sac in the abdominal cavity of a guinea pig; they also isolated the toxin. But repetitions of their experiments by other observers have not been successful. On the other hand, the results of Buchner on cholera plasmin, in which the toxin was extracted by grinding and pressing the germ cells, support the intracellular hypothesis; and we may conclude that the most reliable evidence shows that the cholera spirillum does not produce a powerful soluble toxin, but that its toxin exists as an integral part of the cell, from which, through plasmolysis and digestion, it may be set free.

The ferments.—Another necessary consideration in preparing a cholera prophylactic was the action of the enzymes of the cholera spirillum, their effect upon the protoplasm of their own cells, and the effects of the digestive products thus formed. The cholera vibrio, under certain conditions, produces at least four of these enzymes, the diastatic (Bitter), the inverting

[1] According to Gotschlich and Weigand, after two days only 10% (at the maximum), and after three days only one % (at the maximum) of the organisms present at the end of the first 24 hours were still alive.

(Fermi), the rennin-like (Schoffer), and the peptonizing (Bitter). The work of Gotschlich and Weigang and that of Conradi have shown that the rapid dying-off of cholera spirilla in agar cultures after 24 hours results from the action of degenerative products formed autolytically in the culture. This digestion is probably due to the peptonizing ferment.

PREPARATION OF THE PROPHYLACTIC.

With these two considerations in mind, (1) that the cholera toxin exists as an integral part of the bacterial cell, and, (2) that it is set free after the death of the organism, probably partly through the action of its own proteolytic enzyme, which is not destroyed at 60°, I endeavored to determine whether the other immunizing substances (agglutinin and bacteriolysin), as well as the toxin, could not be separated from the bodies of the bacteria by a process of autolytic digestion.

Accordingly, cholera spirilla from races known to possess good peptonizing powers were placed in an aqueous solution, carefully killed by heating, and digested at 37° C. It was then found that the cholera receptors were set free in the fluid in great abundance, a fact easily demonstrable after filtration, since such filtrates possessed the power of binding uni- and ambo-ceptors (agglutinins and bacteriolysins) in a cholera immune serum, as well as the ability of giving rise, after injection into rabbits, to the appearance of toxic symptoms, and in the case of the ultimate recovery of the rabbit, to the entrance of antitoxic, bactericidal, and agglutinative substances into the blood serum. Such a filtrate, therefore, immediately recommended itself as a trial substance for preventive inoculation. It was prepared in large quantities after the following manner:

The surfaces of large flat-sided flasks (after the pattern of Kolle), filled with agar, were sprayed with 20 hours' broth cultures of the organism, and the flasks put aside for 20 hours at 37° C.[1] The growth was then suspended in sterile water and placed in a sterile flask at 60° C. for from one to 24 hours. The mixture was afterwards put at 37° C. for from two to five days, and finally filtered through a Reichel candle.

In certain experiments the milky fluid overlying the sediment of the

[1] Cultures of 20 hours' duration were always used, on account of the rapid death of the organisms in older cultures. Fresh beef was employed in the preparation of the media. The agar at the time of use had an alkalinity of −1% to phenolphthalein. This gave a sufficient alkalinity to the aqueous suspensions of the organisms to bring about a favorable action of the proteolytic ferment.

bacteria at the bottom of the flask was poured off and the latter crushed and subjected to hydraulic pressure of 600 atmospheres. Later the extracts from these pressed organisms and the original aqueous solution, previously decanted, were together passed through the filter.

EXPERIMENTS WITH THE PROPHYLACTIC.

INTRAVENOUS INOCULATION.

Prophylactic I.—Intravenous inoculation of four rabbits with from two to three c.c. of a fluid obtained in the above manner from the virulent strain, in which, however, the organisms were heated for only one hour at 60° C., caused the death of all the animals. It was thought advisable therefore to weaken this toxic action by a more prolonged heating of the organism, in order that bactericidal and agglutinative immunity might be studied in the inoculated animals.

Rabbit series No. 2.—For this series of rabbits, the suspension was placed at 60° C. for 24 hours, and digested for two days at 37° C., before passage through a Reichel filter. For the sake of comparison, both stems, "Virulent" and the "Avirulent," were treated in this way. One cubic centimeter of each filtrate represented the number of receptors obtained from two loops of the living organisms. The rabbits were of about the same average weight (1,500 grams) as those used in *Series No. 1.* Each animal was inoculated intravenously with one c.c. of the filtrate (equal to two loops). After eight days they were all killed by bleeding and the bactericidal and agglutinative values of their blood sera were determined.

With the intravenous injection into rabbits of one c.c. of virulent Prophylactic I, there were obtained sera showing an agglutinative value with the virulent stem of about 3.3 and 2.5 mg., and with the avirulent one of about 2.0 and 1.66 mg., and a bactericidal value against the former strain of about 0.09 and 0.08 mg. However, in the rabbits inoculated with the avirulent prophylactic the sera were not of nearly so great a value, showing an agglutinative worth of only about 10.0 to 3.3 mg., and a bactericidal one of 1.1 to 0.5 mg.

This series of experiments suggested that by the use of the method of autolytic digestion a better bactericidal immunity was obtainable with the virulent organism, and that the immunity acquired was within certain limits directly dependent upon the virulence of the stem used in the preparation of the virus. The value of the sera obtained from the animals inoculated with the

virulent prophylactic also offered encouragement for a more extensive trial of this method with certain modifications.

Prophylactic II.—Therefore another quantity of the prophylactic was prepared, slight changes being introduced in the method. Twenty-hour agar cultures of the virulent organisms were suspended in sterile distilled water and the suspension divided into three portions, each being placed in a separate sterile flask and kept at 60° C. for 24 hours. The first portion was allowed to digest for two days, the second and third for five. All three were then filtered separately, after which the third was reheated at 60° C. for two hours. In the case of Prophylactic II twice as much distilled water was used in preparing the suspension of the agar cultures as in Prophylactic I. Hence, one c.c. of this filtrate contained only the number of receptors obtained from one loop of the living organisms, and possessed only one-half the strength of Prophylactic I.

Rabbit series No. 3.—Four rabbits were injected intravenously, each with 12 c.c. of this prophylactic, animals Nos. 86 and 87 receiving the portions digested for only two days, animal No. 88 that digested for five days, and animal No. 89 that reheated for two hours at 60° C. after five days' digestion; each animal received the number of receptors obtained from the digestion of 12 loops of the living organisms. After eight days the animals were killed by bleeding and the values of their sera estimated.

The results obtained in this series of experiments gave still greater encouragement for the method, since they showed that by a digestion of five days more receptors were set free from the bacterial cells in an aqueous solution than by one or two days. Animals Nos. 86 and 87, each receiving inoculations of the portions digested for two days, showed an agglutinative value of about 1.25 and 1.43 mg., and a bactericidal immunity of about 0.05 mg.; while animal No. 88, inoculated with the portion digested for five days, showed an agglutinative value of 1.1 mg. and a bactericidal value of about 0.04 mg. Results with No. 89 showed that reheating at 60° C. for two hours had destroyed to some extent the agglutinable substances of the bacteria, as evidenced by a marked loss of agglutinin in the serum, show only 1.6 mg. in value. The substances giving rise to the bactericidal qualities were apparently but little affected by the second heating, since the bactericidal

value of the serum of this animal was between 0.04 and 0.05 mg., only a little poorer than that of animal No. 88 (0.04 mg.).

Prophylactic III and IV.—In order to determine whether more receptors are always set free in the fluid with longer digestion, two more portions of the prophylactic were prepared with both strains, one being digested for five, the other for two days at 37° C. In Prophylactic III sufficient sterile water was used for the suspension of the organisms to make one c.c. of it equal to the number of receptors obtained from two loops of the living organisms; in Prophylactic IV one c.c. of the suspension equaled one loop. Hence, in the inoculation of the rabbits in which Prophylactic IV was employed, double the amount of fluid was injected. The rabbits which received Prophylactic III, digested for five days, all furnished better sera than those injected with the virus of corresponding virulence of Prophylactic IV, digested for only two days. The best serum obtained from the injection of virulent Prophylactic IV was that of animal No. 256, which showed an agglutinative value of 1.4 mg. and a bactericidal one of 0.06 mg.; while the best obtained with Prophylactic III was from animal No. 192, showing an agglutinative limit of one mg. and a bactericidal value of 0.04 mg. In other words, these experiments indicate that even when corresponding amounts of the digested organisms in solution were injected, the animals receiving the more concentrated virus furnished the better serum. However, the differences in value of the sera are so slight that, unless constant, they might be explained by natural variation in the animals used. From these experiments it seemed that sufficient data had been obtained toward securing a good agglutinative and bactericidal serum by means of a protective prepared after this method, and that its success for intravenous injections was assured.

SUBCUTANEOUS INOCULATIONS.

The next step, then, was to discover what degree of immunity could be obtained when the prophylactic was injected subcutaneously. Accordingly, experiments were made with both strains, the inoculations being performed subdermally in rabbits. In such a series of experiments it was found that after the subcutaneous injection of five c.c. of the most favorable virus, sera were

obtained with an agglutinative value of from 2.5 to 1.6 mg., and a bactericidal value of from 0.14 to 0.1 mg. These results were regarded as very favorable.

EXPERIMENTS WITH THE DRIED PROPHYLACTIC.

In several instances the prophylactic was evaporated in a vacuum at 38° C. and then pulverized, after which it was redissolved in normal salt solution, and injected into rabbits both intravenously and subcutaneously in varying amounts. While a distinct loss in the potency of the prophylactic thus treated is evident, nevertheless, a very good agglutinative serum and bactericidal immunity were obtained. Animal No. 167, inoculated intravenously with 10 mg. of the powder obtained from virulent Prophylactic II, produced, after eight days, a serum which agglutinated in dilutions of 10 mg., and showed a bactericidal reaction in dilutions of 0.25 mg. Animal No. 187, inoculated subcutaneously with five mg. of the powder obtained from virulent Prophylactic III, showed after one week a serum of a weak agglutinative value of 10 mg. and a bactericidal one of 1.6 mg.

STUDY OF THE LOCAL AND GENERAL REACTION FOLLOWING THE INOCULATION OF THE PROPHYLACTIC.

In general, it may be said that the animals apparently suffered little from the injection of the prophylactic, even when large amounts were employed. Usually they showed a rise of temperature of one, rarely two, degrees during the 36 hours immediately following the inoculation. A number of rabbits were treated subcutaneously with each separate lot of the prophylactic, for the sole purpose of observing the local reaction. Even when large amounts of the virulent prophylactic (five c.c.) were used for subcutaneous inoculations no suppuration ever occurred, and induration was rarely observed. Usually after 24 hours there was no trace of a local reaction visible. These animals showed a slight and transitory rise of temperature of one or two degrees.

As regards retention of immunity, it may be stated that several animals were killed from three to six months after inoculation, when an examination of their blood sera still demonstrated a high agglutinative and bactericidal reaction.

COMPARISON OF THE IMMUNITY PRODUCED BY THE VIRULENT AND THE AVIRULENT PROPHYLACTIC.

In comparing the immunity obtained by the use of the virulent and the avirulent prophylactic, we see that on the whole the results already referred to are borne out. It will be recalled that in one case the ratio of bactericidal immunity between the animals treated with the virulent prophylactic and those treated with the avirulent one varied between about $3\frac{1}{2}$:1 and 12:1. In another series the sera obtained from the animals inoculated with the virulent prophylactic showed a bactericidal value from about $5\frac{1}{2}$ to 12 times as great as that obtained from the injection of corresponding amounts of "Avirulent." In other cases the animals of the "Virulent" series showed sera from 6 to 15 times as great in value as those of the "Avirulent" ones. With subcutaneous inoculation the proportion is from 8 to 11 times as great; and with the dry prophylactic the value is from $1\frac{1}{3}$ to 4 times as great. The results obtained with the dry prophylactic are certainly not so accurate as those with the fluid, on account of the manipulations to which the powder was subjected; and since they are not in accord with all the other numerous experiments, in which the liquid prophylactic was employed, they need not be considered in this comparative consideration. With this exception the results here reported are in harmony with those which have been obtained by other observers who have employed for inoculation strains of the killed organism of different virulence; namely, that the immunity obtained is within certain limits directly proportional to the virulence of the inoculated strain.

Upon comparing the immunity obtained by the intravenous injection of the prophylactic injection into rabbits with that produced in the same manner by the inoculation of the living organisms, we see that by the injection of one c.c. of the virulent prophylactic (representing the number of receptors obtained from two loops after two days' digestion), there is occasionally obtained a serum nearly equaling in bactericidal and agglutinative properties that produced by the intravenous injection of one-half a loop of the living virulent organisms. By a single intravenous injection of six c.c. of the prophylactic (obtained from 12 loops after five days'

digestion), a serum of far greater value was produced, namely, one agglutinating in dilutions of 1:900 to 1:1000 (one mg.) and showing a bactericidal value as high as 1:24,000 (0.04). Therefore, by a judicious use of this method of autolytic digestion a means is offered us of producing by a single intravenous injection into rabbits a serum of greater bactericidal and agglutinative value than could be produced through the employment in the same manner of either the killed or the living organisms. It is known that an agglutinative value of one mg. and a bactericidal one of 0.04 mg. are not usually to be obtained by the single injection of cultures of either the killed or the living cholera vibrios, even when the inoculation is repeated.

EXPERIMENTS WITH THE PROPHYLACTIC ON GUINEA PIGS.

The following experiments show the effect of the prophylactic on guinea pigs, and also the comparative value of the protection furnished by the virulent prophylactic and that furnished by the injection of living organisms.

Guinea pig series No. 1.—From 6 to 12 animals were inoculated intraperitoneally and subcutaneously with varying amounts of Prophylactics I, II, III, and IV, and of dried Prophylactics II and III respectively, redissolved in normal salt solution. The dose varied from one to five c.c. From 7 to 10 days after the injection of the prophylactic, the animals received intraperitoneally either 5 or 10 times the fatal dose of the living virulent cholera strain. When the "Virulent" prophylactic was employed, the animals were invariably protected; but when the "Virulent" one was used in small amounts of one to two c.c., the guinea pigs sometimes succumbed to the subsequent injection of the living organisms. A few of the animals which received large amounts (five c.c.) of the virulent prophylactic intraperitoneally succumbed, evidently on account of its toxic effects. Upon autopsy no injection of the vessels or hemorrhages at the point of inoculation, such as are always found when death occurs from the inoculation of the living or the dead organisms, were observed. Neither were there any hemorrhages in the serous surfaces of the peritoneum. The most noticeable lesions in these cases consisted of a marked edema of the abdominal walls with some flakes of fibrin over the liver. Microscopically it was observed that an extensive desquamation of the epithelial cells had occurred. The contents of the abdominal cavity were sterile. It is to be noted that one to two c.c. of the thick material, consisting of the débris of the bacteria which accumulated at the bottom of the flask in the manufacture of the prophylactic, and remains behind on the filter in the form of an emulsion, after the prophylactic is passed through, when injected intraperitoneally, causes the death of guinea pigs, in which are found postmortem the most extensive local reactions at the point of inoculation and

throughout the abdominal cavity, consisting of hemorrhagic areas, injection of the larger vessels, and corrosion of the tissues. These effects evidently are caused by toxic substances forming a constituent of the bacterial membrane not soluble in aqueous solution after autolytic digestion, and which have probably little to do with the production of the true immunity against the disease.

Guinea pig series No. 2.— Four large guinea pigs of about the same weight were chosen. Two were inoculated intraperitoneally with 1 to 15 loops of the living virulent organisms and two with five c.c. of the virulent Prophylactic IV. One of those which received the living organisms was very sick for 24 hours following the inoculation. After six days all the animals were reinoculated intraperitoneally with two loops of the living virulent strain. The two which had received the prophylactic previously, lived; the other two died.

Evidently the protection furnished the guinea pigs by the prophylactic was greater than that furnished by the previous injection of 1 to 15 loops of the living organisms, a supposition also borne out, and in a more striking way, by comparing the immunity obtained by the intravenous injection of the prophylactic into rabbits with that obtained by the injection of the living organisms. From the foregoing it is evident that the virulent prophylactic forms a reliable and certain means of protecting guinea pigs against the subsequent injection of multiple fatal doses of the cholera spirillum.

STUDY OF THE TOXIC ACTION OF THE PROPHYLACTIC.

As stated previously, in a few preliminary experiments made with the intravenous injection into rabbits of the virulent prophylactic, if the organisms had been killed by only a very brief period of heating and then allowed to digest themselves, the animals all succumbed to the inoculation. In order to study carefully the agglutinative and bactericidal value of the sera of the inoculated animals, an attempt was made to weaken the toxic action by prolonged heating at 60° C. This heating apparently had the desired effect, as the rabbits then usually survived the inoculations. In order to study this toxic action more closely, a prophylactic was prepared by killing the organisms within a very brief period, digesting at 37° C., grinding, submitting them to a pressure of about 600 atmospheres, and finally filtering through a Reichel candle. Varying quantities of the prophylactic were then injected intravenously into rabbits. The results show that two

c.c. of virulent Prophylactic V prepared after this manner, when injected into rabbits, caused the death of these animals within 24 hours. Four and five c.c. injected in the same way produced death in a much shorter time. However, the animals sometimes recovered from the injection of one c.c., and were afterwards immune. With virulent Prophylactic VI, in which the filtration was performed with a coarser Berkefeld filter and the bacteria subjected to a more thorough crushing process, even one to two c.c. caused the death of rabbits. In the animals which succumbed to the inoculation, the kidneys were found, upon postmortem examination, to be swollen, and showed other evidences of parenchymatous nephritis. The mesenteric vessels were deeply injected, the liver congested and swollen. The lungs showed patches of congestion, hemorrhages, and in one or two cases small pneumonic areas. A few of the animals showed hemorrhages in the peritoneal surface of the small intestine.

Heating the organism at 60° C. evidently destroys most of the primary poison, or, at any rate, converts the toxin into toxoid, since it is necessary, in order to bring about the death of the guinea pigs, to inject intraperitoneally relatively large amounts (three to five c.c.) of the heated prophylactic. The presence of a toxoid would probably be more desirable in a human prophylactic than that of the unchanged toxin, *i. e.*, the toxoid, through the presence of its haptophore group (the toxophore group being lost) is still able to produce antitoxin in the inoculated body, without unfolding its general poisonous effect. Such a result we are able to obtain from the injection of our cholera prophylactic. It was found that three c.c. and even two c.c. of the serum of a rabbit (No. 422), which had previously been inoculated with two c.c. of the virulent heated Prophylactic V, protected other rabbits against two or three times the intravenous dose fatal for these animals; while two c.c. of human serum, obtained from an individual previously inoculated subcutaneously with three c.c. of virulent Prophylactic V, was capable of neutralizing about four times the fatal dose. In one case one-fifth c.c. of the serum of animal No. 423 (previously inoculated subcutaneously with five c.c. of virulent Prophylactic V) protected a rabbit against about four times the fatal dose of the toxin. In

all of these experiments the prophylactic and the sera were mixed immediately before inoculation. The control rabbits without serum died; this being true also in cases in which equal amounts of normal serum were added to the prophylactic before injection. When smaller quantities of the serum were employed, the death of the animals always resulted, nor would two c.c. of the immune serum protect against any higher doses of the toxin. It is admitted that such antitoxic values of the sera, obtained from animals previously inoculated with the prophylactic, are not high; but it is hoped that better antitoxic properties can be produced when more improved methods are employed to extract the intracellular toxin.

In certain experiments recently performed, in which the digested bacteria were ground before filtration in a mortar with fine quartz sand and infusorial earth, a much greater toxic effect on the guinea pigs was observed, these animals dying from intraperitoneal injections of one-half to one c.c. of such a fluid. Judging from my own experience in this respect, it would appear that the most advantageous method for the extraction of the intracellular toxin of the cholera spirillum would be the one which MacFadyen has recently applied with the same end in view to the typhoid bacillus, *i. e.*, the bacteria are ground at the temperature of liquid air, the disintegration having occurred under conditions which precluded the possibility of chemical change. A combination of the method of autolytic digestion which I have described and the method of MacFadyen for the extraction of the toxin might perhaps furnish a more ideal prophylactic for Asiatic cholera, the mixture of the products of these methods being carefully heated at such temperature as to change the larger portion of the toxin into toxoid. My experiments show that with the modified and extracted toxin no local reaction, similar to that produced by the living or the killed cholera organisms, is obtained, even when sufficient amounts of the former are injected to cause death. The problem, therefore, which confronts us, is the extraction of the toxin in larger quantities.

From a few preliminary experiments, already performed with crude apparatus, it would seem that, with the appliances and the methods recommended by MacFadyen, such production should

be more successful. My experiments throw little light on the nature of the structure of this intracellular toxin. That the haptophore group is identical in structure with that of the soluble toxins of diphtheria and tetanus bacilli would appear doubtful.

EFFECT PRODUCED UPON THE TOXIC ACTION OF THE PROPHYLACTIC BY HEAT OR BY ITS PRESERVATION WITH CERTAIN CHEMICALS.

One series of experiments was made[1] showing the effect of boiling upon the toxic action of the prophylactic when injected into rabbits. From these experiments it appeared that the toxic action was destroyed by a temperature of 100° C., since, after the prophylactic had been thoroughly boiled, neither an intravenous injection of one c.c. nor one of two c.c. caused the death of the rabbits inoculated with it; while control animals receiving one c.c. of the unboiled prophylactic always died. Control animals receiving two c.c. of peptone solution of the same specific gravity as the prophylactic were unaffected. Animals which received the boiled prophylactic were apparently but little disturbed by the inoculation, and their blood showed practically no agglutinative action with the virulent strain. Each successive heating of the prophylactic at 60° or over affects unfavorably the toxic as well as the agglutinative substances. However, by a careful heating at 60° for 15 minutes the toxin is apparently not entirely destroyed.

Several weeks' preservation of the prophylactic in chloretone at room temperature resulted in a loss of toxic power and a further change of the toxin into toxoid. After the preservation of the prophylactic for three months in chloretone neither one c.c. nor even two c.c. of it, when injected intravenously into rabbits, caused the death of these animals; though the injection of the latter amount produced illness with a rise of temperature of about two degrees. On comparing these results with those obtained by the use of fresh prophylactic, it is shown that formerly one-half c.c. of this prophylactic brought about death, and that one or two c.c. were always fatal. However, the agglutinable substance of the prophylactic, preserved for three months in chloretone, remained apparently unchanged in both its groups; since the same relative

[1] In connection with the following experiments I wish to express my thanks to my assistant Mr. Charles B. Hare for much aid.

amounts of agglutinin were produced in the sera of the rabbits inoculated with the old prophylactic as were formed in those of the rabbits inoculated with the fresh one, values as high as one mg. being obtained. The bactericidal values of the sera of the animals were not investigated. There was no reason to suppose that any loss in bactericidal power would be found in these sera, as it is well known that the substances giving rise to the bacteriolysins are not so labile as those which produce the agglutinins.

The preservation of the prophylactic with 0.5 carbolic for a long period of time gave practically the same effect as that produced by chloretone, a weakening of the toxic action, so that three c.c. of the carbolized prophylactic, as against one and two c.c. of the freshly prepared, were required to produce death in a rabbit. However, the substances giving rise to the agglutinins and bacteriolysins were apparently not unfavorably affected by this process.

On account of the unfavorable effects of these substances, it has usually been our practice so to handle the prophylactic in its manufacture that it is received from the filter into the sterile tubes, thus making unnecessary any further sterilization either by heat or by the addition of chemicals. With a prophylactic kept sterile and at the temperature of the ice-box, I have obtained very good results five months after its preparation. Of course, the toxic action becomes weaker after a short period of time, and this process gradually increases, owing to the still further change of toxin into toxoid.

HUMAN INOCULATIONS.

After studying the effects of the prophylactic upon animals, it was also desirable to ascertain its action upon human beings. With this end in view, a number of individuals have been injected from time to time with varying amounts (one to five c.c.) of the virulent prophylactic. The inoculations have been made deep into the muscles of the arm. In these cases the local reaction was never very marked. There was usually soreness on pressure in the region of the inoculation, lasting for about 24 hours, and occasionally a slight reddening of the overlying skin was observed. None of the patients have complained of much pain. No sup-

puration has ever been observed, and in fact it may be said that the local reaction is very slight. Following the inoculation there was generally a rise of temperature of from one to three degrees (Fahrenheit), which subsided in from 24 to 48 hours. Headache lasting for a few hours was occasionally complained of. Unfortunately for a further trial of the method, there has not been sufficient cholera present in the city or in the provinces during the past nine months to warrant the introduction of a general inoculation of the people against this disease; nor has there been any opportunity to observe, from an entirely practical standpoint, the immunity of the inoculated. The fact, therefore, that no cases of cholera have occurred among those receiving the prophylactic shows nothing in regard to the value of the method; since it is doubtful to what extent the inoculated have been exposed to the disease. However, it has been demonstrated that the blood sera of the inoculated individuals, both white and native, acquire protective substances. The sera of none of them, before inoculation, caused any agglutination of the virulent organism in dilutions of 1:20 (50 mg.) or any bactericidal action in dilutions of 1:50 (20 mg.). The blood was drawn from one of the veins of the arm one week after the inoculation and after the separation of the serum the value of the latter was determined. From these experiments it appears that three or four c.c. of the prophylactic furnished the best serum, namely, one having an agglutinative value of from 4 to 2.5 mg. and a bactericidal one of from 0.33 to 0.25 mg. These sera are ten times more potent than those obtained by Kolle from the subcutaneous injection of either the living or the killed cholera spirilla in human beings. Kolle's best sera showed a bactericidal value of from 3 mg. to 2.5 mg. They also show a higher value than is usually seen in the sera of human beings who have recovered from an attack of Asiatic cholera, which according to the investigation of R. Pfeiffer may be 10 mg. Therefore, we might presume that a good active immunity had been acquired against the disease by the use of this prophylactic. The antitoxic value of the sera has already been discussed. In case No. 1 two c.c. of the serum protected rabbits against four times the dose fatal for these animals.

We have seen that by the subcutaneous injection of the cholera prophylactic an excellent cholera immune serum can be obtained in human beings. However, the question naturally arises, whether these individuals are protected against intestinal infection with the cholera spirillum. In other words, are they really immune to the disease, Asiatic cholera? Experiments upon animals cannot satisfactorily answer this query. The earlier investigations of Brieger, Kitasato, Wassermann, Haffkine and others upon the point at issue, namely, whether animals could be rendered immune against intestinal infection with Asiatic cholera gave affirmative results. However the more recent work of Pfeiffer, Wassermann, and Sobernheim, demonstrated that immunity in animals against such infection was not to be obtained certainly by the ordinary methods of immunization then in vogue. Since animals are not naturally susceptible to intestinal infection, and since it is only through artificial means that such an infection may be produced in them, evidently the answer to our question can be given only by a practical observation of the individuals inoculated with the prophylactic during a severe and general epidemic of the disease. For this reason it was hoped that a more extensive practical demonstration of the value of the prophylactic could be given before an extended publication of the work was made.[1]

Since, however, the present report has been delayed nearly nine months and as it appears that there will be no greater opportunity in the near future for a more practical test of the prophylactic in these Islands than has already been experienced, it is thought inadvisable to defer for a longer period the publication of the experimental work.

It would seem, however, from the numerous statistics of Haffkine in India and the more recent work of Murata in Japan, that even by the injection of a small amount of the killed organisms a certain degree of immunity against the natural mode of infection is acquired. Therefore, judging from what has

[1] The results of the experimental work were presented to the Manila Medical Society at the meeting of September 7, 1903.

already been said, it is probable that by the use of our prophylactic human beings may acquire a good active immunity against the disease.

CONCLUSIONS.

1. By the autolytic digestion of carefully killed cholera spirilla in an aqueous fluid the receptors become separated from the bacterial cells and may be filtered off in solution.

2. The injection of these free receptors into both man and animals furnishes a means of producing high bactericidal and agglutinative blood sera. The antitoxic value of these sera is however moderate.

3. The subcutaneous injection into man of such free receptors is not only free from any danger, but produces practically no local disturbance and only a slight general reaction.

4. Hence the method is a practicable one for producing a cholera-immune serum in man.

5. It is highly desirable that this cholera prophylactic be given a thorough practical test.

6. It is possible that by the application to the pest bacillus of a slight modification of this method a more satisfactory prophylactic against bubonic plague could be obtained.

Experiments with this end in view have already been commenced in the Biological Laboratory.

The consideration of the comparative results in immunity obtained with the inoculation of the virulent and the avirulent living organisms and with the prophylactics of different virulence will be considered in another paper.

STUDIES IN PHAGOCYTOSIS.*

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INTRODUCTION.

OUR knowlege of the finer mechanisms in the reactions of various infections is as yet in the beginning of its development. This is true especially of such common, clinically and anatomically well-understood infections as those caused by staphylococci and streptococci, pneumonia with its pneumococcemia, and typhoid fever with its bacillemia. The mechanisms, for instance, by means of which the typhoid bacillus overcomes the strong bacteriolytic power of normal human blood to which it seems very sensitive, and thus succeeds in establishing the typhoid infection, are certainly not fully understood. In our previous article on "The Antilytic Action of Salt Solutions and Other Substances"[1] we described certain experiments the results of which seemed to indicate "that in typhoid infections not only is complement used up, in the course of bacteriolysis, which no doubt is going on, but that a certain amount is also neutralized by the soluble products of disintegration of typhoid bacilli." Hence neutralization of complements may play an important rôle in the establishment of some infections in which we have reason to believe, as in the case of typhoid fever, that the bacteriolytic power of the blood is one of the most important means of defense and eventually also of recovery. In the case of streptococcus, staphylococcus, and pneumococcus infections, however, the situation is different because of the absence from the plasma of human and also of other kinds of blood of free bacteriolytic amboceptors with suitable complements for these organisms (with the possible exception in a limited degree of the staphylococcus). For this reason the extensive investigations of the last few years into the mechanisms of bacteriolysis and hemolysis by the serum of normal and immune animals have not afforded so promptly as at

* Received for publication December 27, 1904.

[1] *Jour. of Infect. Dis.*, 1904, 1, pp. 379-403.

first expected the desired basis for penetrating studies in the genesis and cure of these important infections in which general invasion of the blood is so prominent a feature. For these and other reasons of a more positive character we are now in the midst of a significant revival of interest in the relations of the leucocytes and of phagocytosis to the organisms concerned in these infections.* But we do not consider it necessary at this time to enter into any detailed discussion of the importance and scope in these and other infections of phagocytosis, the knowledge of which we owe largely to Metchnikoff and his pupils. Suffice it to point out that the recent work of Wright and Douglas concerning the action of normal plasma (and serum) in phagocytosis has opened the door for a deeper penetration into the nature of the process and the manner in which it may be modified in various directions.

THE OPSONINS OF WRIGHT AND DOUGLAS.

While many investigators have noted that the fluids of the body influence phagocytosis, especially, it was thought, by direct stimulation of the phagocytes, Wright and Douglas[1] were the first to show that phagocytosis by human leucocytes of various bacteria (S. pyogenes albus, M. melitensis, D. pneumoniæ, B. pestis, B. coli, B. dysenteriæ, B. typhosus, B. choleræ asiaticæ, B. anthracis, B. tuberculosis[2]) is directly dependent upon certain substances in the plasma (and in the serum) which they call opsonins.[3]

They concluded that these substances are taken up by the bacteria which then become susceptible to phagocytosis because bacteria digested in serum heated to 60 or 65° C. for 10 to 15 minutes are not taken up by leucocytes, whereas bacteria digested in normal serum and then heated to 60° C. for 10 minutes are taken up readily. The opsonic power of serum is removed by digestion with dead bacteria and by the addition of Daboia venom; it disappears gradually from standing serum, and, as just indicated, it is destroyed by heat to 60 or 65° C.

*In connection with this see G. F. RUEDIGER, "Mechanism of Streptococcus Infection," *Trans. of Section on Path. and Phys. of Am. Med. Assoc.*, 1904, p. 397.

[1] *Proc. of the Roy. Soc.*, 1903, 72, p. 357 and 1904, 73, p. 128.

[2] *The Lancet*, October 22, 1904, 2, p. 1138.

[3] From the Latin *obsono* or *opsono*, "I cater for," "I prepare food for."

It occurred to us that it might prove of interest to study the action of temperature and of different chemical substances upon the bodies concerned in phagocytosis. It was hoped that in this way it might prove possible more closely to analyze the phenomenon itself as well as to learn something of the ways in which the phagocytic power may be modified in those infections in which phagocytosis is thought to be an important means of defense.

TECHNIQUE OF THE STUDY OF PHAGOCYTOSIS *IN VITRO*.

Within certain more or less obvious limitations the phenomenon of phagocytosis lends itself readily to studies *in vitro*. Wright and Douglas employed with satisfaction Leishman's method[1] of measuring the phagocytic power of the leucocytes in their experiments. Equal parts of blood or other fluid containing leucocytes and bacterial suspensions were mixed and exposed to 37° C. for 15 minutes when smears were stained with Leishman's modification of Romanowsky's method and counts made of the bacteria in a certain number of phagocytes. In this way they obtained averages of the numbers of bacteria taken up by the cells.

In our experiments we have followed the same general technical method. In the main we have used defibrinated blood, but in the case of blood of the rabbit and of the guinea pig it often becomes necessary to add leucocytes from pleural exudate because the defibrinated blood of these animals contains so few polymorphonuclear leucocytes. In certain experiments suspensions of washed leucocytes have been made in serum and in 0.85 % NaCl solution. In general a definite quantity of blood or of leucocytic suspension or of a mixture of these and various salt solutions, and an equal quantity of bacterial suspension, are introduced by means of finely graduated pipettes into small test-tubes which are then incubated at 37° C. for one hour when smears are made and stained with Leishman's stain. The number of bacteria in at least 20 leucocytes are counted in each preparation and the averages of the counts so obtained are shown by the figures in the tables.

[1] *Brit. Med. Jour.*, Jan. 11, 1902, 1, p. 73.

PRELIMINARY EXPERIMENTS.

The results of our preliminary experiments with few exceptions corroborate fully the principal observations of Wright and Douglas upon the influence of human serum on phagocytosis of many bacteria by human polymorphonuclear neutrophile leucocytes. We have found further that similar conditions obtain in the case of phagocytosis by the leucocytes of various animals such as the guinea pig, rabbit, dog, goat, white rat, and horse. The leucocytes of the dog and guinea pig, like those of man, lend themselves especially well to the study of the mechanism of phagocytosis.

Table I shows the phagocytic power, numerically expressed, of the polymorphonuclear leucocytes in the defibrinated blood of these animals under the conditions of our experiments. The

TABLE I.

PHAGOCYTOSIS BY LEUCOCYTES IN DEFIBRINATED BLOOD.

Organisms	Human leucocytes	Guinea pig leucocytes	Rabbit leucocytes	Dog leucocytes	Goat leucocytes	White rat leucocytes	Horse leucocytes
Streptococcus 300[1]	24	50	20	50	30	40	50
Streptoc. 381 P [2]	23	0	0.5	25	..	5	..
Streptococcus 104[3]	20	2	0	13	0	0	4
Pneumococcus	0	0	0	0	0	..	0.1
Staphyloc. aureus	27	50	12	13	12	9	..
B. typhosus	5	6.5	5	11	15	13	7.5
Micrococcus X[4]	1.5	4	3	7.6	..	..	28

[1] Isolated from the heart's blood of a fatal case of scarlet fever; non-virulent for rabbits and guinea pigs.

[2] Isolated from the pericardium of a fatal case of scarlet fever; kills guinea pigs and rabbits in doses (ascites broth cultures) of 0.5 % of body-weight.

[3] Isolated from abscess in guinea pig; kills guinea pigs and rabbits in doses of one c.c. or less of 24-hour ascites broth or serum broth cultures.

[4] An unidentified micrococcus non-virulent for rabbits.

absence of phagocytosis in the case of some of the organisms cannot be discussed at this time further than to say that we are now engaged in further studies, the results of which we hope may throw some light upon the problems thus presented. In the case of organisms with such variable physiological properties as the pneumococcus, we believe that the results shown in Table I should not be applied offhand to other strains, especially in view of the contrary results of Wright and Douglas.

On account of the pronounced variation in the susceptibility of various organisms to phagocytosis, it would seem not unlikely that we have here another means of differentiation that may prove useful.

In the case of anthrax bacilli it is difficult if not impossible to count the number of bacilli taken up by the leucocytes. The preparations from the experiments made in the manner outlined with normal human, dog, and goat leucocytes show, however, that practically every polymorphonuclear leucocyte is actively engaged in phagocytosis.

That phagocytosis under these circumstances depends on the action of something in the serum on the bacteria is shown (1) by the absence of phagocytosis on the part of washed leucocytes suspended in salt solution and mixed with untreated bacteria (a fact emphasized by Wright and Douglas, and observed by us in a large number of instances without a single exception), and (2) by good phagocytosis on the part of similarly treated leucocytes mixed with bacteria first digested with normal serum, then washed and suspended in salt solution. Hereafter we shall speak of bacteria so treated as sensitized bacteria. That leucocytes have a special affinity for sensitized bacteria is seen from the fact that in the presence of such bacteria and carmin granules the leucocytes show a marked preference, so to speak, for the bacteria. As is shown in the following Table II various serums may serve to sensitize a non-virulent streptococcus for phagocytosis by human leucocytes:

TABLE II.

PHAGOCYTOSIS BY HUMAN LEUCOCYTES OF BACTERIA SENSITIZED WITH ALIEN SERA.

Human leucocytes	(defibrinated blood)			+	Staphylococcus aureus					22.0
" "	washed, in NaCl solution			+	"	"				1.2
" "	" " " "			+	"	"	treated with human serum			10.0
" "	(defibrinated blood)			+	Streptococcus 300					22.0
" "	washed, in NaCl solution			+	"	"				1.0
" "	" " " "			+	"	"	treated with human serum			14.0
" "	" " " "			+	"	"	" "	guinea pig serum		12.0
" "	" " " "			+	"	"	" "	rabbit serum		14.0

NOTE.—We have found that in order to obtain the same degree of phagocytosis in different experiments it is important that approximately the same number of bacteria be present. In the experiment above many cocci were undoubtedly lost during the process of sensitization and washing.

The phagocytic power of the leucocytes in the pleural aleuronat exudates of the dog, guinea pig, and rabbit is materially increased

after centrifugating and resuspension in serum or defibrinated blood. This indicates that the fluid of the blood contains more opsonin than the exudate. Streptococcus 300 is readily sensitized by normal horse serum for phagosytosis by human leucocytes.

We have not been able to sensitize with human serum a virulent streptococcus so that it is taken up by washed guinea pig leucocytes. This streptococcus is readily taken up by normal human leucocytes, but not by the leucocytes of normal guinea pigs. The interesting problems presented by this observation are reserved for further consideration in connection with the study of phagocytosis by leucocytes of immunized animals. Of course, the possibility of different opsonins even in the same species must be considered.

At low temperatures—1 to 4° C.—bacteria are not sensitized so rapidly by far as at 35 to 37° C.

Table III also shows the influence of serum upon phagocytosis in a striking manner. In this experiment each tube contained 0.3 c.c. of suspension of washed guinea pig leucocytes and falling quantities of guinea pig serum, enough 0.85% NaCl solution being added to make 0.6 c.c. in each case, and 0.5 c.c. of a suspension of Streptococcus 300.

TABLE III.

THE QUANTITATIVE EFFECT OF SERUM ON PHAGOCYTOSIS.

Quantity of Serum	Number of Cocci Taken up
0.2 c.c.	23.4
0.1 "	19.2
0.05 "	14.0
0.025 "	7.5
0.0125 "	2.2
0.006 "	1.5
0.003 "	0.7
0.000 "	0.0

THE EFFECTS OF TEMPERATURE ON OPSONINS IN NORMAL SERUM.

According to Wright and Douglas, bacteria digested with serum heated to 60 or 65° C. for 10 to 15 minutes are not taken up by leucocytes, whereas bacteria digested with normal serum and then heated to 60° C. for 10 minutes are taken up freely.

Our own experiments show that the power to sensitize streptococcus 300 for phagocytosis by washed corpuscles is lost on heating human, rabbit, and guinea pig sera to 54 to 56° C. and dog serum to 58 to 60° C. for 30 minutes. Low temperatures down to 46° C. materially lessen the power of all these sera to sensitize streptococcus 300 for phagocytosis by washed homologous leucocytes.

We have found that when cocci, once sensitized, are exposed for 30 minutes to the temperature at which the serum used for sensitization is inactivated, or preferably 3–4° higher, the cocci are not taken up to any extent either by washed leucocytes or by leucocytes in defibrinated blood (Table IV). Evidently the substance—opsonin—taken up from the serum by the receptors of the cocci becomes changed in such a manner that it not only no longer renders the cocci fit for phagocytosis, but actually prevents the cocci from taking up new opsonin.

Digestion of cocci in serum inactivated by heat does not interfere with their phagocytes by leucocytes in fresh serum, indicating that in the heated serum there no longer is opsonin capable of union with the leucocytes, and heating non-sensitized cocci to 56° C. and higher does not prevent them from becoming sensitized and taken up by leucocytes.

TABLE IV.

PHAGOCYTOSIS OF HEATED STREPTOCOCCI, SENSITIZED AND NON-SENSITIZED, BY HUMAN LEUCOCYTES.

A. Washed Leucocytes.	
Sensitized streptococci (300)	16
" " " heated to 58° C. 30 min.	4.2
" " " " " 60° " " "	2.5
Non-sensitized " "	0.1
B. Leucocytes in Defibrinated Blood.	
Sensitized streptococci (300) heated to 58° C. 30 min.	4.6
Non-sensitized " " " " " " " "	12.6
" " " " " 60° " " "	12.5
" " "	15

It appears as if opsonin, like toxins and complements, possesses two groups of molecules, one haptophore whereby it attaches itself to the bacterial receptors, and one, which may be called the opsoniferous group, whereby is effected in the bacterium some change, physical or chemical, that is necessary for phagocytosis. We may say that when sensitized cocci are heated, the

opsoniferous group is largely inactivated, but as the bacterial receptors remain occupied by the haptophore group, the bacteria are prevented from taking up new opsonin. In accord with Ehrlich's nomenclature, opsonin, the opsoniferous group of which is destroyed or inactivated may be termed opsonoid. So far we have not obtained indications that any amboceptor is concerned in the sensitization of bacteria for phagocytosis by the leucocytes of normal animals.

Whether opsonins under suitable conditions may give rise to the production by animal and bacterial cells of antiopsonins, and whether certain organisms, virulent and otherwise, are protected from phagocytosis by lack of suitable receptors or by the production of antiopsonins or by other means, singly or combined, are problems upon which work is now in progress.

It certainly is of great interest that Ehrlich's lateral chain theory should prove useful in making clear to us certain phases of the complex mechanism of phagocytosis, the physical and chemical laws of which are not wholly understood. The interdependence of the "humoral" and cellular forces in the reactions set up by bacteria receives here a striking demonstration of no little interest when we recall the conflicts between the humoral and cellular theories of immunity.

THE EFFECT OF SALT SOLUTIONS AND FORMALIN UPON PHAGOCYTOSIS BY LEUCOCYTES OF NORMAL ANIMALS.

For the purpose of studying the effect of salt solutions upon phagocytosis by leucocytes of normal animals a number of experiments have been made. These experiments the results of which are given in Tables V and VI were made as follows: Freshly drawn defibrinated blood was mixed with an equal quantity of $\frac{m}{8}$ solutions of the various salts used; whenever less of the $\frac{m}{8}$ solutions was added, the deficit was made up by means of 0.85 5 % NaCl solution. These mixtures were then placed at 37° C. for 30 to 60 minutes, when an equal quantity of suspension of 24-hour growths of the bacteria used was added and the tubes returned to the incubator for one hour more. Smears were made and the number of bacteria taken up by the leucocytes determined as described in the foregoing. The tables show that practically every

TABLE V.

THE ANTIPHAGOCYTIC ACTION OF $\frac{m}{8}$ SALT SOLUTIONS AND OF FORMALIN.

LEUCOCYTES	MICROBE	NaCl	$CaCl_2$		$BaCl_2$		$SrCl_2$		$MgCl_2$		K_2SO_4		$NaHCO_3$		$Na_3C_6H_5O_7$		$Na_2C_2O_4$		$K_4Fe(CN)_6$		FORMALIN 1-2000	FORMALIN 1-5000	
		0.2	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.1
Human	Staph. aureus	26	16.5	17	1	20	18.6	23	10	18	13	12	13	15	12	12	12	17	2	16	3	11	18
Human	Strept. (300)..	17	3.4	11	0	5.8	2	13	0	13	8	8.3	5.6	8.5	12.5	10.7	10	10	0	10	3	12	14
Human	B. typhosus ..	7.6	8	12	0	11	10	10.6	12.4	8.8	3.9	5.7	0	2.8	3 8	4	1.8	4.5	2.2	6.4	0	1.5	2.7
Dog	Staph. aureus	17	7.4	14	10	12.4	16	18	9.5	11	15	17	2	14	2.8	10	4	12	1	7	2	7	7.5
Dog	Strept. (300)..	21	12	17.5	12	19	17.5	22	18	20	17	21	10	17	13.5	20	7	21	0	11.5	1	11	20
Dog	B. typhosus ..	12	6.7	7.5	3	4	5	7	2.5	7	3	4	1	4.6	1.5	5	3	3.6	0	5	1.5	6	6
Guinea pig..	Staph. aureus	9	0	2	0	3	2	6	3	4.5	5	7	0	2.5	0	2	0	2	0	2	0	2	3
Guinea pig..	Strept. (300)..	33	13	18.6	3	11	13.5	18.5	20	27	26	28	15	22	2	11	2	9.5	0	4	0	0	6
Guinea pig..	B. typhosus ..	6.5	0.5	3	0	1.5	2.5	4	3.5	4.7	3.5	5	1.5	3	0	0	0	0	0	0	0	2	2

TABLE VI.

ANTIPHAGOCYTIC ACTION OF $\frac{m}{8}$ SALT SOLUTIONS AND OF FORMALIN UPON HUMAN LEUCOCYTES IN PRESENCE OF ANTHRAX BACILLI.

DEFIBRINATED HUMAN BLOOD + SALT SOLUTION + SUSPENSION OF ANTHRAX BACILLI	PHAGOCYTOSIS (50 Leucocytes Counted)	
	Phagocytosis	No Phagocytosis
Blood 0.3 c.c. + $\frac{m}{8}$ solution NaCl 0.3 c.c. + suspension 0.6 c.c.	50	0
" 0.3 " + " " $CaCl_2$ 0.3 " + " "	0	50
" 0.4 " + " " " 0.2 " + " "	20	30
" 0.3 " + " " $BaCl_2$ 0.3 " + " "	0	50
" 0.4 " + " " " 0.2 " + " "	3	47
" 0.3 " + " " $SrCl_2$ 0.3 " + " "	0	50
" 0.4 " + " " " 0.2 " + " "	43	7
" 0.3 " + " " $MgCl_2$ 0.3 " + " "	13	37
" 0.4 " + " " " 0.2 " + " "	25	25
" 0.3 " + " " K_2SO_4 0.3 " + " "	46	4
" 0.4 " + " " " 0.2 " + " "	50	0
" 0.3 " + " " $NaHCO_3$ 0.3 " + " "	0	50
" 0.4 " + " " " 0.2 " + " "	15	35
" 0.3 " + " " $Na_3C_6H_5O_7$ 0.3 " + " "	2	48
" 0.4 " + " " " 0.2 " + " "	10	40
" 0.3 " + " " $Na_2C_2O_4$ 0.3 " + " "	0	50
" 0.4 " + " " " 0.2 " + " "	25	25
" 0.3 " + " " $K_4Fe(CN)_6$ 0.3 " + " "	3	49
" 0.4 " + " " " 0.2 " + " "	26	24
Blood 0.3 c.c. + Formalin 1-2000 0.3 c.c. + suspension 0.6 c.c.	0	50
" 0.4 " + " " " 0.2 " + " "	10	40

salt used, as well as formalin, materially reduces the amount of phagocytosis as compared with the amount obtained in the control experiment with 0.85 % ($\frac{m}{6.8}$) NaCl solution. In many cases there has been produced a complete suspension of phagocytosis, notably in the case of certain experiments with $BaCl_2$, $NaHCO_3$, $Na_2C_2O_4$, $Na_3C_6H_5O_7$, $K_4Fe(CN)_6$, and formalin. Attention must be called to the fact that it is rather difficult to count accurately typhoid bacilli situated within leucocytes; in many cases the bacilli seem to break into fragments each of which may be mistaken for a complete bacillus. In the case of anthrax bacilli (Table VI) no attempt has been made to count the bacilli within leucocytes, and for obvious reasons. We believe that in this instance the counts of leucocytes with reference to their being engaged in phagocytosis or not give an adequate idea of the influence of the salts studied, and of formalin, which is found to be about the same as that shown by Table V. $MgCl_2$ and K_2So_4 seem to have less antiphagocytic action than the other salts, occupying in this respect the same relative position as in our tables showing the antilytic action of salt solutions.[1]

MODE OF ACTION OF ANTIPHAGOCYTIC SALTS.

The question how these salts (and formalin) hinder phagocytosis, whether by their action on the leucocytes, the cocci, or the serum, is an interesting one. In order to throw some light upon the antiphagocytic mechanism we have made certain experiments the results of which appear to indicate that the serum is the principal point of attack. If the salts, in the dilutions used, had a direct toxic action on the leucocytes one would not expect any phagocytosis on the part of leucocytes that had been suspended for one to two hours in the salt solutions, then centrifugated out and resuspended in normal serum. The following experiment shows, however, that leucocytes digested for one and a half hours in $\frac{m}{8}$ solutions of calcium chloride, potassium ferrocyanide, and trisodium citrate and then transferred again to normal serum take up fully as many streptococci as those treated in the same manner with 0.85% solution of sodium chloride:

[1] HEKTOEN AND RUEDIGER, *loc. cit.*

Defibrinated guinea pig's blood, to which are added leucocytes from a pleural exudate, is centrifugated, the serum withdrawn and replaced by 0.85% NaCl solution; 0.5 c.c. of this suspension of corpuscles is added to four c.c. of solutions of NaCl, $CaCl_2$, $Na_3C_6H_5O_7$, and $K_4Fe(CN)_6$ respectively and the tubes placed at 37° C. for one and a half hours. The cells are now centrifugated out, washed once in NaCl solution and resuspended in 0.3 c.c. of guinea pig serum, making in all about 0.5 c.c. of suspension to which is added 0.5 c.c. of streptococcus (300) suspension. The tubes are again incubated one hour when stained preparations are made and the bacteria taken up by the leucocytes counted with the following average result for each leucocyte:

Leucocytes treated with			NaCl	solution	= 15.1
"	"	"	$CaCl_2$	"	= 11.8
"	"	"	$Na_3C_6H_5O_7$	"	= 16.8
"	"	"	$K_4Fe(CN)_6$	"	= 15.8

The average number of cocci within the leucocytes in the $CaCl_2$ tube is a little smaller than in those from the NaCl tube, but the difference is so small as to fall within experimental error.

Digestion of cocci for half an hour in salt solutions (NaCl, $CaCl_2$, $Na_2C_2O_4$, $K_4Fe(CN)_6$), and then removing them by centrifugating, and again suspending them in NaCl solution does not hinder their sensitization to the usual extent by dog serum.

The following experiment shows that $\frac{m}{8}$ solutions of various salts have a marked inhibitory effect on phagocytosis when added to defibrinated blood before adding bacteria (see also Tables V and VI). If, however, the bacteria added to the mixtures are previously treated with normal serum and washed in NaCl solution nearly the same number of organisms are taken up by leucocytes as in a mixture of defibrinated blood and bacterial suspension alone. This indicates again that the inhibitory salts have no toxic effect upon the leucocytes in the mixtures.

Two sets of tubes are prepared, each containing 0.3 c.c. of defibrinated human blood and 0.3 c.c. of NaCl, $CaCl_2$, $Na_3C_6H_5O_7$, $Na_2C_2O_4$, and $K_4Fe(CN)_6$ solutions respectively. The tubes are placed at 37° C. for one hour and then there is added to one set untreated streptococci (300), to the other set sensitized streptococci (*i. e.*, streptococci digested with human serum at 37° C. for 30 minutes, then washed and suspended in NaCl solution); the tubes are again incubated for one hour, when smears are made and the number of bacteria taken up counted. The result is shown in Table VII.

TABLE VII.

THE EFFECT OF SALT SOLUTIONS UPON PHAGOCYTOSIS OF NON-SENSITIZED AND SENSITIZED STREPTOCOCCI.

$\frac{m}{8}$ Salt solutions	0.3	Phagocytosis of Non-Sensitized	Phagocytosis of Sensitized
Defibrinated blood	0.3	Streptococci	Streptococci
Streptococcal suspension	0.5		
NaCl		12	10
$CaCl_2$		0.5	10.8
$Na_3C_6H_5O_7$		1	9.7
$Na_2C_2O_4$		0.9	7.1
$K_4Fe(CN)_6$		2	7.7

As stated the results of this experiment practically demonstrate that the salts do not owe their antiphagocytic action to any direct toxic effect upon the leucocytes. On the contrary they indicate that the salts act upon the serum, *i. e.*, upon the opsonin which is prevented from so changing the cocci as to make their phagocytosis possible. This being the case it should not be possible to sensitize bacteria to any extent in mixtures of serum and antiphagocytic solutions. And experiment does show that bacteria digested for half an hour in mixtures of serum and $\frac{m}{8}$ solutions of $CaCl_2$, $Na_3C_6H_5O_7$, $K_4Fe(CN)_6$ (as well as in mixtures of serum and formalin—1:2000 in NaCl solution—and of serum and $\frac{m}{1}$ solutions of KCl and NaCl), and suspended in 0.85% NaCl solution are not taken up nearly so well by washed leucocytes as cocci that are sensitized in the usual way.

Human serum 0.2 c.c. and 0.4 c.c. of solutions of NaCl, $CaCl_2$, $Na_3C_6H_5O_7$, $K_4Fe(CN)_6$ respectively are mixed and placed at 37° C. for one-half hour when 0.5 c.c. of a thick suspension of streptococci (300) is added and the incubation continued for half an hour longer; the cocci are now centrifugated out, resuspended in NaCl solution 0.4 c.c. and 0.4 c.c. of a suspension of washed human leucocytes added to each tube. The tubes are again incubated at 37° C. for 45 minutes when smears are made and the bacteria taken up counted with the following result:

Washed leucocytes	+	cocci	treated	in	serum	and	NaCl	-	7.0
"	"	+ "	"	"	"	"	$CaCl_2$	-	0.8
"	"	+ "	"	"	"	"	$Na_3C_6H_5O_7$	-	0.85
"	"	+ "	"	"	"	"	$K_4Fe(CN)_6$	-	0.9
"	"	+ unsensitized cocci						-	0.

We wish to state that the identical results cannot always be obtained with leucocytes and serum from different individuals and from different species, but in all our experiments there has been noted a more or less marked diminution in phagocytosis of cocci treated in serum-salt mixture as compared with the controls.

The same experiment as the previous one, but with anthrax bacilli, dog serum and dog leucocytes, human serum and human leucocytes, gave the results shown in Table VIII, 50 leucocytes being counted with reference to their being engaged or not in phagocytosis:

TABLE VIII.

PHAGOCYTOSIS OF ANTHRAX BACILLI TREATED IN MIXTURES OF SERUM AND $\frac{m}{8}$ SALT SOLUTIONS.

WASHED LEUCOCYTES + TREATED ANTHRAX BACILLI	PHAGOCYTOSIS (50 Leucocytes Counted)			
	Human Serum and Leucocytes		Dog Serum and Leucocytes	
	+	0	+	0
Washed leucocytes + bacilli treated in serum and NaCl	38	12	45	5
" " + " " " " " " $CaCl_2$	1	49	17	33
" " + " " " " " " $Na_3C_6H_5O_7$	5	45	4	46
" " + " " " " " " $Na_2C_2O_4$	1	49	0	50
" " + " " " " " " $K_4Fe(CN)_6$	0	50	1	49
" " + untreated bacilli	4	46	8	42

From the further fact that bacteria remaining unsensitized in serum-salt mixtures are readily sensitized in fresh serum we conclude that the antiphagocytic salt solutions neutralize or bind the opsonin in such a manner that it cannot act upon the bacteria to the full extent. There is always some phagocytosis in the mixtures of blood and salt solutions, and some cocci are sensitized in the serum-salt mixtures even when relatively large quantities of salt solutions are used. When sensitized cocci are added to the mixture of blood and salt solutions the number of cocci taken up by the leucocytes in the tubes containing $CaCl_2$, $Na_3C_6H_5O_7$, $K_4Fe(CN)_6$, etc., is somewhat smaller than that taken up in the tube containing NaCl. At first sight these facts appear somewhat puzzling, but they are easily understood if we assume that the compounds formed by the union of opsonin with salt, or one of the ions of the salt, and opsonin with bacteria are dissociated, that is, that the reactions are reversible. All of the opsonin in a mixture of salt and serum may have united with the salt, but when cocci are added to the mixture there is established an equilibrium between opsonin and salt on the one hand and opsonin and bacteria on the other and some bacteria will necessarily be sensitized. Similarly, if a mixture of blood and salt

contains a slight excess of salt and sensitized cocci are added to this mixture some of the bacteria will be deprived of their opsonin when equilibrium is established between opsonin and salt on the one hand and opsonin and bacteria on the other, and therefore the number of bacteria taken up by the leucocytes in such a mixture is smaller than might be expected.

SUMMARY.

In closing we wish to emphasize the following points:

1. Phagocytosis of many bacteria by the leucocytes of various normal animals, including man, is dependent upon the presence in the plasma of special substances designated by Wright and Douglas as opsonins.

2. The opsonins become attached to the bacteria which then for unknown reasons become susceptible to phagocytosis.

3. The opsonins in the blood of one species may sensitize bacteria for phagocytosis by the leucocytes of a different species.

4. Opsonins are thermolabile substances of a constitution analogous to that of toxins and complements in that they seem to have two groups, haptophore and opsoniferous; by heating sensitized bacteria the opsoniferous group appears to be destroyed, but the inactive opsonin (opsonoid) by saturating the receptors of the bacteria prevents further sensitization by fresh serum.

5. Like complements opsonins may be neutralized or bound by various salt solutions ($CaCl_2$, $BaCl_2$, $SrCl_2$, $MgCl_2$, K_2SO_4, $NaHCO_3$, $Na_3C_6H_5O_7$, $Na_2C_2O_4$, $K_4Fe(CN)_6$) and other substances, *e. g.*, formalin, so that they cannot act upon bacteria. Antiphagocytic action of this nature may be an important factor in the establishment and progress of various infections, especially those caused by streptococci, pneumococci and other microbes in the destruction of which phagocytosis is an important factor.

The
Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. II *March 1, 1905* NO. 2

STUDIES IN THE FREQUENCY, LOCALIZATION, AND MODES OF DISSEMINATION OF TUBERCULOSIS, WITH SPECIAL REFERENCE TO ITS OCCURRENCE IN THE LYMPH NODES AND DURING CHILDHOOD.*

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INTRODUCTION.

From November, 1896, to February, 1897, a bill (which later became a law) concerning "measures against tuberculous diseases," drawn up by Holmboe and Klaus Hanssen, was discussed

*Received for publication January 3, 1905.

in the Medical Society of Christiania. The bill was based mainly on the theory of inhalation as the principal mode of origin of tuberculosis. In the discussion the frequency and the modes and routes of infection were much dwelt upon and opinions varied considerably in regard to the origin of the disease. While some men considered inhalation as the only mode of origin, others advocated infection by way of the intestinal tract as the most frequent source. Dr. Antvord took a special position, based on a thorough investigation of statistics, and claimed that nearly two-thirds of all tuberculous patients contract the disease in childhood, but he gave no further explanation as to how pulmonary tuberculosis of adults originates.

No agreement as to the modes and routes of infection was reached. Personal investigations on a large scale were presented by Dr. J. Bugge, but these concerned only tuberculosis of the lungs and bronchial nodes, mainly in adults. Professor A. Johannessen treated tuberculosis of childhood very thoroughly from the clinical side. Tuberculosis in children from the point of view of morbid anatomy, on the other hand, was not especially considered, although one would expect such a point of view to suggest itself, on account of the greater simplicity of the conditions in children, where, *a priori*, it might be supposed the occurrence and distribution of the disease could be more easily cleared up. Attention would naturally be drawn to the frequency in children of tuberculosis of lymph nodes, especially the cervical nodes—a fact long established by clinical experience.

Some time previously, and while this discussion was going on, I had, as assistant in the Pathological Institute, performed a large number of autopsies upon children. I had noted the frequent, often very extensive, and sometimes even general, tuberculosis of the lymph nodes, and its primary occurrence, especially in relation to pulmonary tuberculosis, in children. In this manner my interest in tuberculosis of childhood was aroused. I therefore decided to give the subject more thorough attention, and I have occupied myself with it ever since, though with many interruptions. In the following years (1898–1900) I performed as many autopsies upon children as possible, with a view to demon-

strate the frequency of tuberculosis in the various groups of lymph nodes. I paid especial attention to the cervical nodes, and, in connection with them, to the tonsils.

During a sojourn in Leipzig in the summer of 1898, I also occupied myself chiefly with this topic, material being kindly given to me from the Pathological Institute of Leipzig by Professor Birch-Hirschfeld, and from the hospital for children conducted by Professor Soltmann (prosector, Dr. Seiffert). However, time only sufficed for the investigation of about 30 autopsies in children.

Having become professor of pathology in the University of Christiania in the summer of 1900, thus securing material of my own, I was enabled to take up these investigations more extensively and to commence a systematic and broader study of the entire subject. It is upon the results of these studies, made from January, 1901, to April, 1904, that my conclusions are mainly based. I shall also, to some extent, consider my earlier investigations (1898–1900) because they help to elucidate certain points in regard to tuberculosis in children, although it is insufficient in other respects to form the basis of conclusions.

I have placed the line between child and adult at the completion of the 15th year. Others, e. g. Nägeli, have placed it at 18, which appears to me rather late, and it is common to place it at 10 or 12. To draw the line at the 15th year, as is perhaps most generally done, seems to me more correct, as in regard to morbid anatomy, the behavior of tuberculosis before and after this age varies considerably, though, naturally, there is no sharp distinction. It is just at and after the 15th year that the transitional forms are seen, lymphatic tuberculosis passing towards the background and becoming superseded by pulmonary tuberculosis in its ordinary adult form. Such a line must, however, necessarily be arbitrary.

It was originally my plan to occupy myself with tuberculosis of childhood only, and especially its primary localizations, with particular reference to the lymph nodes. However, the opportunity to investigate a large number of autopsies in adults has led me to consider those aspects of the occurrence and evolution of

tuberculosis in adults which are directly related to the disease in children. Thus the scope of my work has become much wider, and I trust that in this manner observations of importance have been added. The facts concerning adults over 15 years which I have attempted to elucidate are the following:

1. The frequency of tuberculosis in adults in general, as arrrived at from statistics.

2. Lymphatic tuberculosis in the adult, its frequency, localization, and connection with other tuberculous affections, especially with tuberculous infection in childhood.

3. Primary localizations outside the respiratory tract, especially in the digestive tract.

It is self-evident that all these questions are too broad to be discussed in all their bearings. They are here treated only to the extent suggested by my various observations, and in so far as they have a relation to the main subject, tuberculosis in childhood.

MATERIAL.

My material has been obtained principally from the Pathological Institute, from private autopsies, and from the Municipal Hospital of Christiania. I have, so far as possible, availed myself of accessible literature, but as it is impossible to include everything written on the various aspects of tuberculosis, and as extensive reviews may be found in numerous recent articles, I have considered a complete review of the literature concerned, superfluous. I shall refer principally to the larger recent publications, and to the Scandinavian, particularly the Norwegian, literature, which is relatively little known outside of Scandinavia. Otherwise I shall refer to large collective reviews, such as those of Strauss, Cornet, Hildebrandt, Lubarsch and Ostertag's *Ergebnisse der allg. Path. u. path. Anat.*, Baumgarten's *Jahresberichte*, etc.

METHODS EMPLOYED.

My work has concerned principally the lymph nodes of children. At each autopsy particular attention was directed towards the three chief groups, those of the neck, of the chest, and of the abdomen. In the neck, together with the mucous membrane of

the throat and the tonsils, the superficial and deep groups of nodes, both superior (submaxillary) and inferior (superficial and deep cervical, and supraclavicular), were systematically examined at every autopsy in children. Most frequently changes were observed in the submaxillary nodes. In general the use of the term cervical nodes is meant to signify the submaxillary groups. In the thorax, the nodi bronchiales, nodi hili pulmonares, nodi tracheales, and nodi mediastinales (sometimes also the nodi axillares) were examined. In the literature most frequently the term "bronchial node" is used collectively; here, so far as possible, the special groups have been kept apart. The lungs, of course, were always carefully examined. In the abdomen, the mesenteric nodes were examined (before separation from the intestines); also the retroperitoneal and sometimes the inguinal nodes, and, of course, the entire intestinal tract. At first the investigation was mainly directed to the cervical nodes, the throat and tonsils, especially in the examinations made before 1901. This material, therefore, has been somewhat incompletely elaborated.

The investigations consisted of (1) autopsy with detailed gross examination of the various groups of lymph nodes, (2) inoculations of animals, (3) microscopic examination.

All inoculations were made into guinea pigs. Under aseptic precautions several small nodes or pieces of several large ones were placed under the skin of the right side of the back and the wound sutured. In positive cases swelling and caseation in the nodes of the right groin regularly appeared; afterwards the spleen, liver, and retroperitoneal lymph nodes were attacked, and finally the bronchial and cervical lymph nodes and the lungs. Different guinea pigs were regularly inoculated with material from the cervical, intrathoracic, and mesenteric nodes respectively, whenever swollen nodes could be demonstrated. As a rule the animals were killed after two months. It might be added that only a single case of spontaneous tuberculosis has been observed in our animal barn in the past year.

Microscopic examination was made of the remains of nodes from which inoculations had been made and of neighboring swollen nodes. Nodes from the three chief groups were always

examined and in the largest numbers possible (as a rule from 3 or 4 to 10 or 12). Sections were taken at different levels; serial sections were not made as it would have taken too much time in such an extensive investigation. The microscopic examinations were made as soon as the inoculated animals were killed. In case of positive results, several sections were examined, a number of specimens always being stained for tubercle bacilli.

Occasionally, as recommended by English writers, the fresh juice expressed from swollen lymph nodes was spread upon cover glasses and examined for tubercle bacilli.

It is self-evident that in cases where the autopsy disclosed macroscopic evidences of tuberculosis of the lymph nodes, the examination was confined to the other group of nodes. It is self-evident, too, that the autopsies were conducted with the object also in view of demonstrating tuberculosis in other organs than the lymph nodes.

SUMMARY OF RESULTS OF AUTOPSIES IN CHILDREN.

Before giving my own results I will first briefly review some of the previous statistics concerning tuberculosis in children, with special attention to the publications of last year and to the Scandinavian literature.

An extensive work on tuberculosis in children, mainly of clinical interest, by Abelin[1] appeared in Sweden in 1882. His material was very abundant, comprising 21,932 infants under one year of age, observed from 1842 to 1881, and it has been studied with special reference to miliary tuberculosis. There were 421 deaths from tuberculosis out of a total of 5,410, a mortality of 7.7 per cent from tuberculosis varying from 11.2 per cent to 4.8 per cent in different years. Abelin does not go into details as to the anatomical localizations, though he declares that the bronchial nodes constitute the focus where the "contagion" is at first taken up and from which it spreads.

Medin[2] gives a further analysis of the same material. The 411 deaths deaths from tuberculosis in the first year, detailed information of which was available, were distributed as follows: First month, 5; second, 7; third, 47; fourth, 69; fifth, 82; sixth, 49; seventh, 31; eighth, 30; ninth, 27; tenth, 30; eleventh, 15; twelfth, 16. Ordinary tuberculous meningitis was found in 44 children, and tubercles in the meninges in 23. The points of origin, as a rule, were the lymph nodes and the respiratory tract, more rarely the digestive tract.

The statistics of Biedert[3] include 36,148 children. Of these 1,932 (5.3

[1] *Nord. Med. Arkiv.*, 1882, 14, p. 1.

[2] *Nord. Med. Arkiv.*, 1883, 15, p. 1.

[3] *Jahrb. f. Kinderheilk.*, 1885, 21, p. 158.

per cent) suffered from tuberculosis. Of 1,308 children who died within the first year, 68 per cent had tuberculosis.

The material of Froebelius[1] consisted of 91,370 infants in the St. Petersburg "Findelhaus," 65,680 of whom were ill, with 18,569(?) deaths and also 18,581 autopsies. Among the latter the cause of death was tuberculosis in 416, or four per cent of the total number of infants, 2.2 per cent of the deaths, and 2.5 per cent of the autopsies. Twenty of these died in the first two months, while 396 (95.1 per cent) were two months old or more. (Forty-six, two months; 73, two and one-half months; 54, three months; 55, three and one-half months; 56, four months; 26, four and one-half months; 20, five months; and only 56 six months or more).

In 902 autopsies in children under 15, dead of acute infection, Babes[2] found tuberculosis in 288 (31.9 per cent) and always in the lymph nodes. In 93 autopsies performed in 1887, tuberculosis of the bronchial and mesenteric nodes were present in 65. Death was caused by tuberculosis in only 13 of these cases.

From Denmark we have two valuable publications by Geill[3] on lymphatic tuberculosis in children, its frequency and seat (1888 and 1890). Of 902 autopsies in children under 15, dead from acute infections, he found tuberculosis in 288 (31.9 per cent) distributed as follows:

Under three years	430 autopsies	89 cases	(20.7%)
3–6 years	334 "	139 "	(41.6%)
6–9 "	98 "	46 "	(46.9%)
9–12 "	35 "	14 "	(40%)
12–15 "	5 "	0 "	

Monrad[4] reports finding tuberculosis in 157 out of 654 autopsies in children (only five cases were latent). In the first year 9.2 per cent of the cases (33 of 356) were tuberculous; in the second year 32 per cent; third to fifth year 46.9 per cent, and fifth to fifteenth year 49.4 per cent.

The work of Müller[5] is important. In 500 autopsies on children in the Munich Pathological Institute death was due to tuberculosis in 150 (30 per cent), and 59 (11.8 per cent) had latent tuberculosis which most commonly was virulent, rarely obsolete.

Hecker's[6] work is a continuation of Müller's statistics. In 700 autopsies in children tuberculosis was the cause of death in 97 (13.9 per cent), and latent tuberculosis was present in 50; hence a total of 147 (21 per cent) with tuberculosis infection. Combining the two sets of statistics we get a mortality of 20.5 per cent. According to the figures of Müller tuberculosis was rare in the first year and then rapidly increased, 41.3 per cent of all cases occurring between the first and fourth year, then decreased, and again increased between 13 and 15. Müller mentions three cases dying in the first year, at twelve, eight,

[1] *Jahrb. f. Kinderheilk.*, 1886, 24, p. 47.

[2] Quoted from BAUMGARTEN'S *Jahresberichte.*

[3] GEILL, C., *Hosp.-Tid.*, 1888, 3 R., 6, pp. 249, 278; *Bibliothek f. laeger*, 7 R., 1890, 1, p. 725.

[4] *Hosp.-Tid.*, 1902, 10, p. 300.

[5] *Zur Kentniss der Kindertuberkulose.* Inaugural Dissertation, 1890.

[6] *Münch. med. Wchnschr.*, 1894, 40, p. 391.

and four months respectively. Hecker 10 cases, one under three months; three, three to six months; and six, six to 12 months old.

From the Pathological Institute of Kiel (Professor Heller) statistics have been published by Schwer, Simmonds, and Boltz.[1]

Schwer:	728	autopsies	in	children.	Death	from	tuberculosis	in 17 %
Simmonds:	576	"	"	"	"	"	"	" 21.6%
Boltz:	1,272	"	"	"	"	"	"	" 13.8%
Total:	2,572	"	"	"	"	"	"	" 16.7%

Of Boltz's 1,272 cases 153 were stillborn and 201 less than four weeks old. None of these died of tuberculosis. Between five and 10 weeks one child died of tuberculosis (which was about one per cent of the total deaths at that age). Between three and five months 8.6 per cent died of tuberculosis; 12 months 8.3 per cent; one to two years 26.8 per cent; two to three years 33 per cent; three to four years 29.6 per cent; four to five years 31.8 per cent; five to 10 years 34.3 per cent; 10 to 15 years 30 per cent. In 1,430 autopsies in children under one year 64 (4.5 per cent) were found to be tuberculous; in 781 one to five years old 230 (29.3 per cent); in 228 five to 10 years 78 (35 per cent); in 162, 10 to 15 years 56 (34.6 per cent).

From later statistics by Heller[2] we learn that in 714 children dead of diphtheria latent tuberculosis was found in 140 (19.6 per cent). A recent collective report from Kiel by Hof[3] shows that 936 (20.1 per cent) of 4,649 children which came to autopsy were tuberculous.

Nebelthau[4] in 1903 collected the material from 17 statistics and found that in 8,770 autopsies in children tuberculosis had been found in 1,796 (20.4 per cent).

In 806 autopsies in children reported by Baginsky[5] tuberculosis existed in 144 (17.8 per cent).

H. Schmidt[6] collected the material from the Pathological Institute of Erlangen for the years 1863 to 1895 and found tuberculosis as the cause of death in 325 children under 10 years, 23 per cent of the total number of this age. In 11 additional cases latent tuberculosis was found.

Racynskyi[7] found a very similar percentage. Of 12,152 children treated in the stationary clinic of St. Ludwig's Hospital for children in Cracow, 3,341 died, 611 (18.3 per cent) of tuberculosis. In the 2,720 other autopsies latent tuberculosis was found in 112 or 4.1 per cent, a strikingly small number. Of 828 who died under one year of age 74 (8.8 per cent) died of tuberculosis; of 624 one to two years 123; of 419 two to three years 118; of 627 three to five years, 126; of 453, five and two years, 125; and of 260, eight and 12 years, 45 died of tuberculosis.

1 *Ein Beitrag zur Kindertuberkulose.* Inaugural Dissertation, 1890.

2 *Münch. med. Wchnschr.*, 1902, 49, p. 609.

3 *Ueber primäre Darmtuberculose nach 15000 Sectionen.* Inaugural Dissertation, 1903.

4 *Münch. med. Wchnschr.*, 1903, 50, pp. 1246; 1300.

5 *Deutsche med. Wchnschr.*, 1902, 28, (Vereinsbeil.), p. 270.

6 *Ueber die Häufigkeit der Tuberkulose in den verschiedenen Lebensaltern.* Inaugural Dissertation, 1897.

7 *Jahrb. f. Kinderheilk.*, 3. F., 1901, 4, p. 67.

Kossel[1] has given an important contribution. Of 286 children under 12, 36 (12.6 per cent) had tuberculosis. The disease was latent in 14 of these cases (all over one and one half year) while 22 had died of tuberculosis. Of 226 under one year 14 (six per cent) died of tuberculosis. Of 60 over one year eight died of tuberculosis while fourteen had latent foci. Of 59 children suffering from other diseases 28 (40 per cent) reacted to tuberculosis (*i. e.*, they had latent tuberculosis).

Nägeli[2] found 15 cases of tuberculosis (17 per cent) in 88 autopsies in children. There were 35 autopsies with six cases of tuberculosis (17 per cent) at the ages of one to five years, 33 per cent tuberculosis at five to nine years, 38.7 per cent at nine to 17 years.

In Norway an extensive statistical work by J. Bugge[3] has appeared. This is of special interest as it only includes cases examined by himself, and because microscopic examination and animal inoculations quite frequently were employed. However, his autopsies in children are comparatively few. Of 21 under one year one had tuberculosis; of 22 between one and nine years 15 (68 per cent), 12 of these fatal, three latent; of 18 between 10 and 19 years (most of them over 15), 16 (89 per cent) had tuberculosis (six died of it, six had latent virulent foci). Hence, in the bodies of 61 persons under 19 years of age tuberculous changes were found in 32 (over 50 per cent).

Various statistics are also found in the English literature. Thus Still[4] in 769 autopsies in children under 12 years found 269 cases of tuberculosis (35 per cent); 43 of the cases were latent; 117 cases (43.4 per cent) occurred before the second year.

In America Hand[5] found 115 cases (34.6 per cent of tuberculosis in 332 autopsies in children. Councilman, Mallory, and Pearce[6] in 220 children dead of diphtheria found latent tuberculosis in 35 (16 per cent). Cohaus[7] in 459 children likewise dead of diphtheria (from Kiel) reports 95 cases of tuberculosis (20.7 per cent), Cronemeyer[8] 60 out of 459 (13.3 per cent).

In a recent discussion in the Berliner medizinischen Gesellschaft, Orth[9] gave his experiences from the Charité for the preceding 15 months. Of 1,558 autopsies 287 were in children under one year. Ten of these (3.4 per cent) had tuberculosis; of 131 cases between the ages of one and 15 years 37 (28 per cent) had tuberculosis, *i. e.*, about 11 per cent tuberculosis in all autopsies in children. In the same discussion Baginsky[10] related that he had observed 1,383 cases of tuberculosis in children, 245 of which (17.2 per cent) occurred in the first year: first month, two; second, seven; third, 23; fourth,

[1] *Ztschr. f. Hyg. u. Infektionskr.*, 1896, 21, p. 59.

[2] *Virch. Arch.*, 1900, 160, p. 426.

[3] *Undersögelser om Lungetuberkulosens Hyppighed og Helbredelighed.* Christiania, 1896.

[4] *Brit. Med. Jour.*, August 19, 1899, 2, p. 755.

[5] *Proc. Path. Soc., Phila.*, 1903, 6, p. 132. Also *Arch. Pediat.*, 1903, 20, p. 427.

[6] *Jour. of the Bost. Soc. of Med. Sci.*, 1900, 5, p. 139.

[7] Cited by COUNCILMAN, MALLORY, and PEARCE.

[8] Cited by COUNCILMAN, MALLORY, and PEARCE

[9] *Berl. Klin. Wchnschr.*, 1904, 41, p. 265.

[10] *Verh. d. Berlin. med. Gesellsch.*, February 10, 1904.

15; fifth, 30; sixth, 34 etc.). Lubarsch[1] states that in 297 autopsies in children under 15 years tuberculosis was found in 63 cases (21.2 per cent).

We come now to my own investigations. At first the results of my work in Leipzig in the summer 1898 will be given. Thirty children came to autopsy; microscopic examinations were made, mainly of the lymph nodes, and especially of the cervical group. Tuberculosis was demonstrated in nine (30 per cent); in five of these as the cause of death (ages eight months, one and one-half, one and one-half, two and one-fourth, and four and one-half years respectively), while in the other four cases it was an accidental finding, in two (six and three-fourths and eight years old) exclusively in the bronchial and hilus nodes and demonstrable macroscopically, in the other two only microscopically (in the one child in the mucous membrane of the throat and cervical nodes, in the other both in the cervical and hilus nodes).

Of the five children dead of tuberculosis, in three the starting point was in the intrathoracic nodes and in the lungs; in the other two there was an almost universal lymphatic tuberculosis which in one of the cases seemed to have commenced in the cervical nodes. In the other 21 children (70 per cent), 11 of whom died in the first year, six in the second, three in the third, and one six and one-half years old, there was no evidence of tuberculosis; in all of them the cervical nodes were examined microscopically, in most of them also the bronchial nodes, but only in about one-fourth the mesenteric nodes. Inoculations in guinea pigs, on the other hand, were not made; for that reason these cases have not been included in the tables to be given later.

As to the investigations made at the pathological institute in Christiania, I shall group those made in 1898–1900 separately as Series I (partly incompletely studied); those made between January, 1901, and February, 1904, and completely worked up, as Series II.[2]

[1] *Fortschr. d. Med.*, 1904, 22, p. 701.

[2] The details of each case cannot be given here, but will be found in my work: "Studien über die HAufigkeit, Lokalisation und Ausbreitungswege der Tuberkulose, insbesondere mit Berücksichtigung ihres Sitzes in den Lymphdrüsen und ihresVorkommens im Kindesalter." *Forh. af Videnskabsselskabet i Christiania*, 1904 (math.-naturvid klasse, No. 8).

In the years 1898–1900 (Series I) 133 children under 15 years came to autopsy. Forty-eight of these (36.1 per cent) were infected with tuberculosis, 85 (63.9 per cent) were free from macroscopic signs of the disease; and inoculations in guinea pigs with lymph nodes, tonsils, etc., gave negative results, as did microscopic examinations whenever made.

In the years 1901–April 1904 (Series II) there were 142 autopsies in children under 15 years. In 69 cases (48.6 per cent) tuberculous infection existed, while in 73 cases (51.4 per cent) no tuberculous changes were found, and inoculations from them were negative. Hence, the number of infected cases is greater in this series, principally because in many cases latent lesions existed which were only revealed by inoculations or microscopic examination.

Combining the two series we have 275 autopsies in children, in 158 of which, *i. e.*, 57.5 per cent, no tuberculous lesions or tubercle bacilli were found, while they were present in 117, or 42.5 per cent.

It will be noted that these figures are very high, especially those of Series II in which nearly half the cases show tuberculous infection, and even if we only consider the total figure of 42.5 per cent they exceed those in nearly all the statistics previously mentioned, in most of which the percentage was in the neighborhood of 20 (Monrad, 24; Hecker, 21; Schwer, Simmonds, and Boltz, 16.4; Baginsky, 17.8; the series from Kiel, 20.1; Nebelthau, 20.4; H. Schmidt, 23; Racynskyi, 22.4; Kossel, 12.6; Nägeli, 17; Orth, 11; Lubarsch, 21.2). Several authors, however, give figures from 30 to 35 per cent.—Babes, 31.9; Geill, 31.9; Müller, 41.8; Still, 35; Hand 34.1.

It is of interest that Bugge, whose material also came from the pathological institute of Christiania, likewise found a high percentage, over 50 per cent. However, his 61 autopsies also included cases between 15 and 19 years of age. He also made thorough microscopic examinations.

The objection might be raised that the high percentages could be accounted for by the source of our material, but the state hospital in Christiania ("Rigshospitalet") from which most of

our material was obtained, admits all classes of patients, and preferably non-tuberculous cases. This is particularly true of the pediatric wards. Private and medicolegal cases were also included in order to avoid any kind of selection. The high figures are not only due to the many fatal cases, but also to a large number in which the presence of latent tubercle bacilli was revealed by animal inoculations. It will also be noticed that numerous instances of tuberculous infection were met with in the large number of children less than one year or even only a few months old which I examined. This is shown by the succeeding tables of autopsies of tuberculous and non-tuberculous children.

TABLE I.

NON-TUBERCULOUS CHILDREN.

	Series I 1898–1900		Series II 1901–April 1904		Total	
0– 3 months	22	42 in first year	25	56 in first year	47	78 in first year
4– 6 "	14		12		26	
7– 9 "	2		11		13	
10–12 "	4		8		12	
1– 2 years	21		10		31	
2– 3 "	6		0		6	
3– 4 "	4		2		6	
4– 5 "	1		0		1	
5– 6 "	0		0		0	
6– 7 "	2		1		3	
7– 8 "	2		1		3	
8– 9 "	2		0		2	
9–10 "	0		1		1	
10–11 "	1		0		1	
11–12 "	0		1		1	
12–13 "	0		1		1	
13–14 "	1		0		1	
14–15 "	3		0		3	
	85		73		158	

It is seen that most of the non-tuberculous children were under two years of age, two-thirds of them even under one year.

In the following table of tuberculous cases latent or obsolete tuberculosis is placed separately, and in Series II also cases in which latent bacilli were demonstrated by inoculations.

TABLE II.

TUBERCULOUS CHILDREN.

	SERIES I—1898-90		SERIES II—1901-APRIL 1904			TOTAL
	Died	Latent and Obsolete Tuberculosis	Died	Latent and Obsolete Tuberculosis	Latent Bacilli	
0- 3 months	3 } 7 in	..	1	..	2 } 18 in	6
4- 6 "	0 } first	..	4	..	3 } first	7
7- 9 "	2 } year	..	0	1	3 } year	6
10-12 "	2	..	2	1	1	6
1- 2 years	4	..	5	..	2	11
2- 3 "	1	..	1	1	3	6
3- 4 "	2	..	..	1	..	3
4- 5 "	3	..	2	..	..	5
5- 6 "	3	..	3	1	..	7
6- 7 "	1	..	1	..	2	4
7- 8 "	5	1	..	1	1	8
8- 9 "	0	1	..	..	..	1
9-10 "	1	1	1	1	..	4
10-11 "	4	2	2	2	1	11
11-12 "	2	..	1	1	..	4
12-13 "	0	3	1	1	..	5
13-14 "	2	1	1	..	..	4
14-15 "	4	..	8	7	..	15
	39	9	33	18	18	117
	48		69			

To summarize: Of 117 tuberculous cases 72 or 61.5 per cent died of tuberculosis; 21 cases (23 per cent) had latent or obsolete tuberculous lesions, while in 18 cases (15.3 per cent) latent tubercle bacilli existed. As before stated, the percentages in Series I probably are too small as seen by comparison with those of Series II. Nevertheless, it may be of interest to compare Tables I and II. After the fourth year the tuberculous cases are seen to predominate over the non-tuberculous ones, and in increasing ratio. Thus, among the 22 children between 14 and 15 years old, 19 had tuberculosis.

On the other hand, if we compare those under four years, most of them are seen to be without tuberculous infection, and here also the relative frequency increases with the age. This progressive increase becomes very plain if we compare conditions in the quarters of the first year, with only 11.3 per cent of tuber-

culous cases in the first quarter, 21 per cent in the second, etc., constituting a good argument against the frequency of congenital tuberculosis, and being in accord with the findings in most other statistics.

TABLE III.

FINDINGS IN CHILDREN UNDER FOUR YEARS OF AGE.

Age	Number of Non-tuberculous Cases		Number of Tuberculous Cases		Total Number Examined
0– 3 months.....	47	98	6	25	123
4– 6 "	26		7		
7– 9 "	13		6		
10–12 "	12		6		
1– 2 years.....	31		11		42
2– 3 "	6		6		12
3– 4 "	6		3		9
4–15 "	17		72		89

However, the number of tuberculous cases in the first year (in 25 of 123 autopsies) is large as compared with the statistics of others. In addition to the 14 fatal cases (four, four, two, and four cases per quarter respectively), it is noteworthy that two cases of latent tuberculosis occurred at this age, and the fact deserves special emphasis that in no less than nine cases in the first year latent tubercle bacilli were demonstrated, while two such cases occurred in the second year and three in the third year. This will be further discussed later.

The statistics of other writers, with much smaller proportion of tuberculosis in the first year, are generally based on heterogeneous material collected from long periods and by different prosectors, without any special effort having been made to demonstrate a tuberculous infection; mention is not made of microscopic examination and of inoculations. Thus Monrad in children under one year of age found tuberculosis in 9.2 per cent (in the second year in 32 per cent), Boltz in 4.5 per cent (from one to five years 29.3 per cent), Racynskyi in 8.8 per cent (in the second year in about one-sixth, third year one-third to one-fourth etc.), Kossel, in six per cent (but in children over one year in 36 per cent), Orth in 3.4 per cent (but in children from one to 15 in 28 per cent). Baginsky noted the frequent

occurrence of tuberculosis in infants a few months old but does not give the percentage at different ages; Abelin in 7.7 per cent, Froebelius in 2.5 per cent of infants.

The cases of latent and obsolete tuberculosis were relatively few, being 27 in our two series combined, or in 9.8 per cent of the 275 autopsies performed, or in 23 per cent of the total 117 tuberculous cases. The number is also small compared to the number of cases in which tuberculosis was the cause of death (72), and particularly small when compared to the 18 cases in which latent bacilli were found. The reason may partly be in the nature of the material which included very few cases of contagious diseases, in which (measles, diphtheria etc.) experience seems to show that latent tuberculosis is most frequent (Babes 31.9 per cent, Müller 11.8 per cent, Kossel 40 per cent, Heller 19.6 per cent, Councilman, Mallory, and Pierce 16 per cent, Cohaus 20.7 per cent, Cronemeyer 13.3 per cent etc.). Another reason lies in the fact that so many of the children in this series had died during the first year in which latent and obsolete forms are known to be rare.

The latent and obsolete tuberculosis was most frequently located in lymph nodes which were caseous, and partly in the lungs, in the latter case, as a rule, together with changes in the thoracic lymph nodes. The lymph nodes within the thorax were found to be infected most frequently (25 times), the cervical nodes and tonsils nine times, and the mesenteric nodes seven times.

Most frequently the diagnosis of tuberculosis could be made macroscopically. However in 12 cases it could only be made after microscopic examination.[1] Thus, in a girl nine years old, fibrous and hyalin tubercles were found in the bronchial nodes. In a girl, 14 years old, with caseous tracheal and bronchial nodes the cervical nodes on histological examination were found tuberculous, and positive results were obtained by inoculations from enlarged mesenteric and retroperitoneal nodes. In a girl, 12 years old, who died of a fracture of the femur, all groups of lymph nodes were somewhat enlarged; histologically, fibrous and

[1] The same was true of two of the nine tuberculous cases in the 30 Leipzig autopsies.

hyalin tubercles and hyalin degeneration were demonstrated in the cervical, bronchial, mediastinal and mesenteric nodes. In a child, four years old, dead of joint and miliary tuberculosis, tubercles were found in enlarged cervical nodes and in the tonsils. The tubercles were partly recent and of usual appearance, partly fibrous and hyalin, and an extensive hyalin degeneration in other parts of the parenchyma indicated a marked regressive tendency of the disease in these nodes. In a boy, 10 years old, all nodes, especially in the cervical group, were enlarged. Negative results were obtained by inoculations from the cervical nodes, but all nodes examined presented extensive hyalin degeneration, and fibrous and hyalin tubercles of typical structure, *i. e.*, a general lymphatic tuberculosis had almost passed away.

On the whole hyalin degeneration in lymph nodes always creates a suspicion of tuberculosis and is a valuable guide in the histological examination. On the other hand, I have not found the so-called large-celled hyperplasia in lymph nodes to be indicative of tuberculosis except in the presence of distinct tubercles. I find no reason to enter into other microscopic details.

Along with the twelve cases where microscopic examination only revealed tuberculosis of the lymph nodes we have numerous and extensive microscopic examinations with negative results, as shown in our series of non-tuberculous children. In 54 of the 73 cases (Series II) with negative gross finding microscopic examination was made of the cervical nodes and tonsils (in 52 cases), of the tracheobronchial nodes (in 27 cases) and of the mesenteric nodes (in 30 cases)—with negative results in all. In 37 of these cases inoculations of guinea pigs were also made and with negative results.

LATENT TUBERCLE BACILLI IN THE LYMPH NODES.

We now come to an important point where my investigations have brought positive results in a direction to which hitherto little attention has been given, namely, *the occurrence in lymph nodes of tubercle bacilli demonstrable by inoculation, without concurrent macroscopic or microscopic changes.*

What do we find in medical literature concerning this subject?

Information is as yet scarce, especially as to autopsies in children, though it appears that recently more attention has been given to the subject. The most important of these investigations, some of which also concern adults, are the following:

Loomis[1] made intrapleural inoculations in rabbits of material from the bronchial nodes of 30 adult bodies free from macroscopic tuberculosis. He claims positive results in eight cases (plus three uncertain ones), but the results are not very convincing as several of the animals are said to have died in 10 or 15 days. The nodes were not examined microscopically.

Nor are Pizzini's[2] experiments unassailable though generally accepted as correct. He inoculated rabbits intraperitoneally and subcutaneously with material from the bronchial, mesenteric, and cervical nodes of 40 adult bodies. In 10 cases the animals died of peritonitis or septicemia. Of the animals inoculated from the remaining 30 cases 11 became tuberculous. In all successful inoculations bronchial nodes had been used, in one also cervical nodes, while no positive results were obtained from the mesenteric nodes. Only four cases (Nos. 9, 10, 22, and 29) are unobjectionable, while in the others the animals died of tuberculosis so early and with such severe changes that doubt is thrown on the reliability of the experiments.

Spengler[3] examined macroscopically and microscopically the bronchial nodes of six children one-half to nine years old. In all cases he found tubercle bacilli in cover glass preparations from the nodes even in cases without gross or microscopic evidence of tuberculosis. Inoculations were not made. The mesenteric and cervical nodes were similarly examined, with negative results.

Perez[4] on the basis of animal experiments, claims that tubercle bacil may remain latent in lymph nodes, and that they rapidly lose their virulence and only give rise to a mild infection. Tubercle bacilli, injected subcutaneously, soon disappeared from the blood and viscera but remained for months in the lymph nodes from which they could be obtained in pure culture. They had lost their virulence.

The interesting experimental research of Manfredi and B. Frisco[5] deals with the latency of microbes in lymph nodes and their immunizing effect. They showed that this latency of microorganisms in lymph nodes is frequent and generally harmless, but may be the starting point of a "cryptogenic infection" of the body, that the virulence of the organisms decreases and an immunizing effect is exerted on the body. They also showed that tubercle bacilli may penetrate the skin or mucous membranes without producing lesions in them, to be retained in the lymph nodes and remain alive a certain time (three to four months). There they undergo a form of granular degeneration and gradually lose their virulence. According to the number and virulence of the bacilli and the power of reaction of the lymph nodes the result is either

[1] *Jour. Am. Med. Assn.*, 1891, 16, p. 98; abstract in *Deutsche med. Wchnschr.*, 1892, 18, p. 756; quoted by Pizzini (*loc. cit.*)

[2] *Ztschr. f. klin. Med.* 1892, 21, p. 329.

[3] *Ztschr. f. Hyg. u. Infectionskr.*, 1893, 13, p. 347.

[4] *Centralbl. f. Bakt.*, 1898, 23, p. 404.

[5] *Centralbl. f. Bakt.*, 1902–03, *Refer*, 32, p. 295.

a mere latent scanty deposit of bacilli, without specific histological changes, or a local eruption of tubercles, or a general infection.

Kälble[1] injected guinea pigs intraperitoneally with emulsions in sterile broth of bronchial nodes from bodies without gross or microscopic tuberculous lesions. Of the 23 animals which did not succumb to suppurative peritonitis two became tuberculous. It was concluded that in these two cases the glands had contained virulent tubercle bacilli without presenting demonstrable changes (one was a man of 41, the other a child of five and one-half years.)

The observations of Walsham[2] are less important. He demonstrated microscopically tubercle bacilli in the bronchial and cervical nodes obtained from autopsies in 26 tuberculous adults and children, and maintains that in about one-fourth of the cases tuberculous infection cannot be recognized merely by the histologic picture, among other reasons because a really tuberculous large-celled hyperplasia readily might be misinterpreted.

The best and weightiest contribution to the study of the occurrence of latent bacilli in lymph nodes is a recent one by Allan Macfadyen and Alfred MacConkey.[3] These observers injected subcutaneously and intraperitoneally in guinea pigs the crushed mesenteric nodes from the bodies of 28 children. The animals were killed after six to eight weeks. Only two of the children were over five years. Eight had various tuberculous lesions. Of the remaining 20 cases positive results were obtained in five, one a still-born infant, the others six months, six months, two and one-fourth years, and eight years old respectively.[4] In two of these cases tuberculosis was present histologically so latent bacilli were really only demonstrated in three (or two) cases. The same authors in a similar manner examined the adenoid vegetations of 44 children, and the tonsils of 34, all with negative result.

In my Series II[5] of 142 autopsies it will be remembered that latent tubercle bacilli were demonstrated in 18 of the 91 cases in which there was no gross nor histological sign of tuberculosis. As stated, no tubercles or tuberculous granulation tissue could be found by painstaking histological examination of the nodes and other parts of the body in these cases.

The objection might be raised that as the nodes used in the inoculations could not also be examined histologically, microscopic lesions might have existed. If so, not only latent tubercle bacilli, but also real latent tuberculosis, would have been present,

[1] *Münch. med. Wchnschr.*, 1899, 46, p. 622.

[2] *Jour. of Path. and Bact.*, 1901, 7, p. 409.

[3] *Brit. Med. Jour.*, July 18, 1903, 1, p. 123.

[4] As to the still-born infant it is also stated that the result of the inoculation from the mesenteric nodes was negative.

[5] Series I cannot be used in this connection on account of the small number of inoculations made.

and the existence of virulent bacilli be of less interest.[1] However, this explanation is hardly correct when we consider the large number of successful inoculations and the thorough histological examination made of adjacent nodes of the same groups. It would be strange if tubercles, if present, would not have been discovered in at least a few of the nodes examined. The mere macroscopic examination by an experienced pathologist is also of great value, though, of course, far from sufficient.

On the other hand, this objection may justly be raised against many of the series of experiments mentioned where inoculations only, and no histological examinations, had been made (see the investigations of Macfadyen and MacConkey).

In order to attempt to demonstrate such latent tubercle bacilli in apparently normal tissue, I have also examined for tubercle bacilli sections from nodes adjacent to those from which successful inoculations were made. Chances were naturally small on account of the probably small number of bacilli present. I have made such controls in 10 cases, with negative result. Bacilli were never demonstrated with certainty; only red granules and rod-shaped bodies.

In these examinations attention was also directed to the so-called "capsules of Schrön," "Keimprodukten" of tubercle bacilli, which d'Arrigo[2] claims to have seen in scrofulous nodes, which findings, however, hardly have met with general recognition. Walsham, however, has demonstrated such involution forms in caseous nodes. But no such globular or oval bodies, which d'Arrigo describes and believes to be products of tubercle bacilli, could be demonstrated.

Perhaps latent tubercle bacilli might have been demonstrated in still more cases if search had been made in cover glass preparations made from the expressed juice, a method apparently successfully employed by several earlier observers (see Spengler, Walsham), but which I have tried only a few times. By this

[1] This objection has been strongly raised against the work of Loomis and Pizzini by Bugge (*loc. cit.*, pp. 50–51), who also has shown that in a node which shows no gross evidence of tuberculosis the disease may be demonstrated by histological examination of one-half of it, and inoculation of the other half give positive result.

[2] *Centralbl. f. Bakt.* I. 1900, 28, p. 481.

method one should be enabled to examine a larger quantity of tissue, and it ought to be borne in mind in future investigations:

It is of great interest to note the age of the children in which latent bacilli were detected, and the groups of nodes from which successful inoculations were made. Both will be shown in the following tables:

TABLE IV.

NUMBER OF CASES SHOWING LATENT BACILLI AT DIFFERENT AGES.

0– 3 months	2	10
4– 6 months	2	
7– 9 months	5	
10-12 months	1	
2d year	3	
3d year	2	
6th year	1	
7th year	1	
11th year	1	
Total	18	

TABLE V.

NODES GIVING VIRULENT BACILLI ON INOCULATION.

Cases from Series II	Age	Nodes
1	2¾ years	Cervical nodes
2	2 years	" "
3	7½ months	" "
4	10 years	" "
5	2½ years	" "
6	14 months	" "
7	3½ months	" "
8	4 months	" "
9	2 months	" "
10	6½ months	Cervical and mesenteric nodes
11	6 months	Cervical nodes
12	5 years	Mesenteric and retroperitoneal nodes
13	9½ months	Cervical nodes
14	1 year	Cervical and tracheal nodes
15	52 days	Tracheal nodes
16	6 months	Cervical, tracheal, and mesenteric nodes
17	6 years	Cervical nodes
18	8 months	" "

It is a striking fact that virulent bacilli were demonstrated most commonly in very young children who also formed the bulk of the autopsy material. Not less than 10 of the 18 cases were in children less than one year of age, three were between

two and three years, and only three over three years. By comparison it will be recalled that of the 73 non-tuberculous children, 56 were less than one year old (25, 12, 11, and 5 in each quarter) and 10 between one and two years; as will also be remembered, the lymph nodes were examined microscopically in 54 cases and inoculations made 37 times.

It is also a noteworthy fact that virulent tubercle bacilli, as a rule, only have been demonstrated in certain groups of lymph nodes, oftenest in the cervical nodes. Thus, the cervical nodes gave positive result in not less than 13 cases, to which one case must be added in which both the cervical and mesenteric nodes gave positive results, and also one with positive results from the cervical, tracheal, and mesenteric nodes. However, it must be borne in mind that in the beginning the work, both as regards inoculations and histological examination, attention was especially directed to the cervical nodes.

Another reason for the selection was the fact that these nodes most commonly were enlarged and thus invited further investigation. In the 73 cases with negative findings the cervical nodes were examined histologically in 52 cases and inoculations from these nodes and the tonsils were made in 34 cases.

In the mesenteric nodes bacilli were found in only three cases; namely, in these nodes alone in one case, together with the cervical nodes in one, and with the cervical and tracheal in one (in a child six months old). This is a small number, and probably too small, as until the middle of 1902 observation was not sufficiently directed toward these nodes, and relatively few inoculations were made from them. It should, however, be remembered that in 21 of the 73 non-tuberculous cases inoculations from the mesenteric nodes had been made and with negative result. Future investigations might advantageously be specially directed to these nodes,[1] the more so as the address of v. Behring on the routes of infection has caused more attention to be given to the digestive tract in recent discussions, and because many facts point to this tract being a more frequent place of invasion than generally supposed.

[1] Such investigations on a rather large scale are being pursued by a Norwegian, Dr. Hans Thue; they are as yet unfinished and unpublished, but have given positive results in a large number of cases.

It is surprising how rarely positive results were obtained from the bronchial, hilus, and tracheal nodes, in which otherwise tuberculous changes most frequently and most easily have been found. There were only three positive results, one from these nodes only (in a child 52 days old), one together with the cervical nodes, and one with the cervical and mesenteric nodes. Negative results were obtained from these nodes in several cases in which inoculations from other nodes, especially the cervical, gave positive result. In the 73 cases free from tuberculous infection, microscopic examination of these nodes was made in 27 cases and inoculations in guinea pigs in 16 cases. One reason why these nodes were less thoroughly examined, especially less than the cervical nodes, is the fact that they are more rarely enlarged in little children. However, as general experience as well as some of the researches on latent tubercle bacilli previously mentioned point to the frequency of tuberculous infection in these nodes these investigations also ought to be continued on a larger scale.

The cases of simultaneous occurrence of latent tubercle bacilli in several or even all groups of lymph nodes are of interest. We shall return to these cases when discussing the localization and points of invasion of tuberculosis, as they ought to be discussed in connection with cases in which tuberculous lesions simultaneously exist in several groups of lymph nodes.

As already mentioned on several occasions, the swelling of the different groups occurs at different times so that most of the groups are found enlarged when the children have grown older. My material must be considered quite well suited to aid in forming an opinion on on this point as it chiefly consists of children dead within the first year or even a few months after birth. It is a noteworthy fact that the cervical and (perhaps somewhat later) the mesenteric nodes first become swollen. This is the general rule. In a given case the cause of death naturally is of great importance. Of the cervical nodes the *nodi cervicales profundi superiores* show a particularly early enlargement (these nodes drain the pharynx, larynx, tongue, etc.). The bronchial, hilus, and tracheal nodes, on the other hand, are involved later. In the

first weeks or months after birth these nodes are not swollen and so small as to be difficult of recognition. Hence, the irritating substances, including the micro-organisms, which cause the swelling of the nodes, appear to reach the nodes in the chest relatively late. This is clearly borne out by the study of cases.

As a result of these investigations we have the demonstration in a comparatively large number of cases, of the presence of latent tubercle bacilli in the lymph nodes, and especially in the cervical nodes. The following questions then arise: *Do these bacilli possess their usual virulence? How long may the bacilli be supposed to have lain latent in the lymph nodes?*

Both questions are extremely difficult to answer. With reference to the first question, it might seem probable that bacilli which are found in lymph nodes and may be supposed to have been there for some time, should be possessed of comparatively little virulence, having produced neither demonstrable changes nor a further infection of the organism.

Contrary to the conception of former times, it may now be considered settled that tubercle bacilli found in the human body as the cause of various tuberculous affections also possess a variable virulence.

Among other reasons, this is also suggested by the various ways in which tuberculosis may appear and develop. Experiments with different strains of tubercle bacilli and their virulence in animals also seem to point in the same direction. Numerous inoculations in animals of tuberculous material of various kinds have also taught me that the time which elapses before the death of the animals from tuberculous infection varies greatly, a phenomenon not otherwise readily explained than by difference in virulence.

Some knowledge of the behavior of the latent bacilli in this respect might perhaps be gained by studying the results of animal inoculations with special reference to the development of tuberculosis in the animals. It would then soon be noticed that the results were not uniform. It is true that most of them died after two months, or were killed at this time, because they gave signs of advanced tuberculosis. But it was not unusual for the

animals not to die until after three or four months, or it was found convenient to kill them at this time because not until then was the existence of the disease definitely established.[1] And sometimes the disease was comparatively very little advanced even when the animal was killed after three or four months. It even happened that some animals remained healthy while others inoculated from the same nodes became tuberculous and died. Such occurrences cannot well be explained except in two ways—either by assuming that the animals have received very few bacilli, which does not appear probable to me, or that the inoculated bacilli, perhaps few in number, were of comparatively slight virulence. To look for the explanation in a variable susceptibility on the part of the guinea pigs seems less reasonable to me; at least little is known about it.

Everything considered, it appears plausible to assume that the latent tubercle bacilli not rarely have shown a less virulence than usual. This also is in accord with the experiments of Perez and of Manfredi and Frisco.

But *how long* may it now be supposed *that bacilli may lie latent in the lymph nodes* or elsewhere in the body? This is a question of the greatest importance, especially so far as the different theories concerning modes of infection in tuberculosis are concerned. The idea of the prolonged latency of tubercle bacilli in the organism is well known. V. Baumgarten, in particular, has always brought it forward when advocating the frequency of intrauterine infection, and believed that the explanation of cases of tuberculosis appearing late in childhood or in adults must be sought in a latency extending over even long periods. And v. Behring, as is well known, has recently advocated the same idea; but he placed the infection in early childhood; then, as it appears from his writings, the bacilli should remain latent in order later, perhaps first in adult life, on some occasion to break out and cause damage. As to a period of latency reaching back to intrauterine life, we shall come back to it in a subsequent chapter. At this time suffice it to remark that there is not

[1] The lesions always appeared in this order: swelling of the inguinal nodes on the right side; then of the retroperitoneal nodes, spleen, and liver; later of the tracheal, bronchial, and cervical nodes, and lungs.

much evidence on which to consider congenitally latent tubercle bacilli as frequent or of practical importance (as is true of congenital tuberculosis in general). Still, in this connection I will also express the opinion that, while I must take issue with v. Baumgarten as to his hypothesis in general, I believe that congenital infection is more frequent than commonly believed, which is also true of latent tubercle bacilli in the lymph nodes.[1] Yet I must assume that most tuberculous infections occur after birth. This, however, does not bring us nearer to a solution of the question of the duration of latency. It is extremely difficult to express anything definite concerning this. The reduced virulence of the bacilli which I have just emphasized might seem to speak for a prolonged latency, if infection in these cases is not due just to bacilli of slight virulence. As is known, animal experiments seem to indicate that the prolonged sojourn of the tubercle bacilli in an animal organism brings about a reduced virulence (Perez, Manfredi, and Frisco).

On the other hand the age of the children appears to point to a brief stay in the organism. We have seen that latent tubercle bacilli most frequently have been demonstrated in the first two or three years, and especially in the first year. If now the microbes have invaded the body after birth, it must be concluded that they have remained latent a relatively short time, some months (at most a couple of years). This also agrees well with the conclusions reached by Perez and Manfredi and Frisco, as they found that tubercle bacilli injected into animals might remain latent (but enfeebled) for several months (three or four).

Against prolonged latency speaks also the fact that latent bacilli comparatively seldom were demonstrated in older children (over three years), in whom one would expect to find them frequently in case they may remain latent for years. Still, it must be borne in mind that the bulk of the children examined belonged in the first two years. Nor does there seem to be any relation between the degree of virulence of the bacilli demonstrated

[1] In only one of the 18 cases is it directly stated that the mother had tuberculosis; so here the possibility of prenatal infection was present.

(judging from the results of inoculations in animals) and the age of the child: bacilli of ordinary or high virulence were found in children over two years in whom bacilli of low virulence should have been expected, and conversely, tubercle bacilli of low virulence were found, particularly in children a few months old.

It is thus seen that my investigations do not support the view that virulent tubercle bacilli may remain latent for years in the body, and particularly in the lymph nodes. Probably this cannot be proven. From what has been stated, at most the conclusion may be drawn that a latency of several months seems likely. I believe, however, that at present a latency of several years cannot be denied, though it is not probable, as little evidence now can be brought in favor of such a view.

It is also the general opinion that a latency of years is neither proven nor probable. This opinion has, among others, been strongly maintained by Cornet. Lubarsch,[1] who otherwise does not entirely reject v. Baumgarten's theory, comes to the conclusion that the assumption of prolonged latency (for years) of tubercle bacilli in non-tuberculous foci, without multiplication of the germs or production of pathological processes, is unproven and in itself little probable.

The results of the numerous microscopic examinations and inoculations of lymph nodes from various regions should also be correlated with clinical observations on the frequency of enlargement of the lymph nodes in children and opinions formed as to its relation to tuberculous infection. In this way the nature and frequency of scrofula again become involved, now a burning question. While most authorities consider scrofula, at least in its most common forms, as identical with tuberculosis, others enlarge the conception of the term scrofula to include almost any form of enlargement of lymph nodes, even if other clinical features of scrofula are lacking. The latter view has been most strongly expressed in Cornet's work on scrofula (1900), where two forms are established, one dependent on infection with tubercle bacilli, another mainly on infections of different nature, particularly with pyogenic organisms. This is not the place to

[1] *Loc. cit.*

take up this question in its entirety.[1] I will call attention only to the most important articles in which attempts have been made to determine clinically the frequency of tuberculosis of lymph nodes in children. In these it is partly taken for granted that swollen lymph nodes mean tuberculous infection, although the authors concerned have not had the opportunity to establish the real existence of the latter, and on this basis conclusions, in part far reaching, concerning the freqency of tuberculosis in children, the avenues of infection, as well at the further infection of the organism with tubercle, are reached. Still these contributions are of great importance.

One of the earliest investigators of the frequency of enlargement of lymph nodes in children is Volland,[2] who also deserves credit for having at an early time brought up for discussion the question of the connection between tuberculous lymph nodes in childhood and a later pulmonary tuberculosis. Volland examined 2,506 persons between the ages of seven and 24 years. Of

628	aged	7– 9	years,	607	had enlarged	cervical	nodes.	96.6	per cent.
724	"	10–12	"	664	"	"	"	91.6	"
722	"	13–15	"	607	"	"	"	84	"
337	"	16–18	"	233	"	"	"	69.7	"
98	"	19–24	"	67	"	"	"	68.3	"

Hence, about 94 per cent of the children between seven and twelve years had enlarged cervical nodes. Volland, however, hesitates to consider all these nodes tuberculous, yet he believes that such infection exists in numerous cases, and on this he bases his theory of infection with tubercle bacilli from skin and mucous membranes by means of dirt and uncleanliness, and here he places the cause of eventual later infection of the internal organs, particularly the lungs.

Neuman,[3] in his review on tuberculosis of the bronchial nodes, also touches on the same question. In 4,883 children from one to nine years old he found a marked chronic swelling (presumably tuberculous) in 309, *i. e.*, 6.38 per cent, and in 2.9 per cent other undoubtedly tuberculous affections. He found most of the cases in the first two years.

Investigations similar to those of Volland have been made by Laser,[4] likewise in school children. He examined 1,216 children, 1,079 of whom had enlarged cervical nodes; at different ages they were found in from 70 to 100 per cent of the cases. Exclusive of other causes of such enlargement (angina, eczema, etc.), he came to the result that in 32.4–58.9 per cent of the cases

[1] Reviews of most recent work of interest on this subject are found in LUBARSCH-OSTERTAG'S *Ergebnisse d. allg. Path. u. path. Anat.*, II, VI, and especially VIII, Abth., 2 (1902).

[2] *Ztschr. f. klin. Med.*, 1893, 23, p. 50; also *Münch. med. Wchnschr.* 1904, 51, p. 87.

[3] *Deutsche med. Wchnschr.*, 1893, 19, p. 202.

[4] *Deutsche med. Wchnschr.*, 1896, 22, p. 500.

tuberculous nodes were present. However, he considers the latter figure rather high as the other etiologic factors are frequently involved. Laser also seeks the source of the infection in inhalation of dust, infection through dirty hands, etc.

In Norway Randers[1] has advocated similar views, emphasizing from clinical experience the frequency of tuberculosis in childhood. In 1899-1901 in the Private Policlinic for Children in Christiania he treated 1,671 new patients; of these 311 were found to be tuberculous, 137 boys and 174 girls. Aside from swollen lymph nodes presumably tuberculous changes in the lungs were found in 99 cases. Most frequently the cervical nodes were affected, regularly also when the axillary, inguinal, and iliac nodes were involved. Tuberculosis of the mesenteric nodes was recorded in 17 cases. Randus also examined 200 boys and about 180 girls in a public school; six boys and nine girls had symptoms of tuberculosis of the respiratory organs, and 182 boys and 160 girls had more or less extensive latent lympathic tuberculosis.

From these investigations it may be concluded that swelling of the cervical nodes is very frequent, and that many clinical da are in favor of the tuberculous nature of this swelling. This result is in full accord with my demonstration of latent tubercle bacilli which were found particularly frequently in the cervical nodes. It may then be considered reasonably certain that lymphatic enlargement, especially cervical, in a large number of cases is due to tuberculous infection, but also, that, in a still larger number of cases it appears to be due to other forms of irritation than tubercle bacilli. And there is also no reason why the tubercle bacilli when demonstrated, might not, at least in part, have been something accidental, in other words, that an enlargement of the lymph nodes previously had existed to which the tuberculous infection had been added.

TUBERCULOSIS IN EARLY CHILDHOOD. CONGENITAL TUBERCULOSIS.

In every study of tuberculosis in children where the probable modes of infection are to be discussed the possibility of the disease being congenital must be taken under consideration. Even if at present only few ascribe much practical importance to this mode of infection, there are, however, prominent pathologists (for instance Baumgarten and his pupils) who still believe in the congenital origin of most cases; and many others, for

[1] *Norsk Mag. f. Laegev.*, 1902, 63, p. 221.

instance Warthin, believe that this mode of infection is of greater importance than commonly supposed.

It is not within the scope of this work to consider this question in its entire breadth; this would also be entirely superfluous as "congenital tuberculosis" has been exhaustively treated in recent years both in monographs and in shorter articles.[1]

I shall only discuss certain phases of this question which come up in connection with my material.

First two cases from the pathological institute of Christiania will be related in which the tuberculosis in all probability was congenital. Ths first case has previously been briefly reported by the late Professor H. Heiberg,[2] the other case I have myself examined.

Case 1 (Professor H. Heiberg's case. Autopsy No. 72, 1874). The child was born at full term (weight 2.5 kg.) and died nine days later. The mother was 33 years old and had had syphilis six years previously; it is not stated whether she also had tuberculosis. The pleura over the left lung was covered with numerous small hyalin tubercles. In the lower part of the left upper lobe was a gelatinous, infiltrated portion of the size of a hazelnut from which purulent fluid could be expressed and in which tubercles were seen. In the lower lobe there was also a pneumonic area of considerable size with numerous tubercles. The right lung presented similar, more advanced changes. There were numerous tubercles both in the mucosa and serosa of the intestines.

Although there are no histological and bacteriological examinations, yet considering the entire postmortem findings together with the definitely expressed opinion of an experienced pathologist of the nature of the lesions, one may assume with certainty that a tuberculous process had been present. Professor Heiberg remarks that it could have been nothing else and adds that "as the process was so far advanced it had probably commenced during intrauterine life."[3]

Case 2 was that of a child, three weeks old, whose father was and still is perfectly well, but the mother had commenced ailing shortly before pregnancy.

[1] Reference may be given to the following: HAUSER, *Deutsche Arch. f. klin. Med.*, 1898, 61, p. 221. CORNET, *Die Tuberculose*, 1899; *Die Scrophulose*, 1900. HILDEBRANDT, *Tuberculose und Scrophulose*. 1902. Review in LUBARSCH-OSTERTAG'S *Ergebnisse d. allg. Path. u. path. Anat.*, 2, 1895; 6, 1899; 8, Abth. 2, 1902. WARTHIN and COWIE, *Jour. of Infect. Dis.*, 1904, 1, p. 140. SCHLÜTER, *Die foetale tuberculöse Infection*, 1902.

[2] *Forh. for det medicinske selskab i Christiania*, 1894, p. 208 (suppl. to *Norsk Mag. for Laegevid*, 1894.)

[3] Unfortunately the organs were not preserved so examination for tubercle bacilli could not be made later.

Early in the course of the latter she developed a dry pleurisy, but later she was quite well and there was then no suspicion of any serious form of tuberculosis. From two to three weeks after delivery, however, signs of extensive pleurisy developed and lasted from two to three months. Afterwards signs of pulmonary tuberculosis appeared, from which disease she died a little over a year after the birth of the child. The child was born at full term, well nourished, and apparently well. The mother nursed it. It showed no symptoms during the first two weeks; then spasms of the glottis appeared, and it died exactly three weeks after birth.

It is to be regretted that the placenta was not examined. The autopsy was made by Professor Peter F. Holst who kindly has placed the material at my disposal.

There was extensive tuberculosis with a large number of tubercles disseminated in various organs, but especially in the lungs where also large consolidated areas were found. The microscopic specimens from the lungs showed an extensive pneumonia with an alveolar exudate rich in cells and fibrin; the exudate here and there showed signs of beginning necrosis. In addition some indistinct tubercles were seen consisting mainly of epithelioid cells and leucocytes and with caseous centers. Everywhere in the tubercles and in the pneumonic exudate enormous masses of tubercle bacilli were present so the specimens in places were entirely red.

Both the extensive and marked changes and the short duration of the life of the child are in favor of intrauterine infection.[1]

We will now consider a little more closely the occurrence of and deaths from tuberculosis in infancy in connection with the question of infection before or after birth. It is evident that the mere occurrence of death from tuberculosis in the first weeks or months after birth must make one think of the possibility of congenital tuberculosis. At what age to draw the line in this respect is a question. Generally cases with death after six months are disregarded, and as a rule attention is only given in this connection to deaths in the first three (or four) months. This is only true on the supposition that it is assumed that tubercle bacilli when once introduced into the body quite soon become active, and that the possibility is not considered that they may remain alive and virulent (but latent) for a considerable period before commencing to exert an influence on the organism. This latter view is the one held by v. Baumgarten, and also maintained

[1] The presence of tubercle bacilli in the blood and internal organs of newborn children (stillborn, or dead soon after birth) has been reported from the pathological institute of Christiania by DR. JENS BUGGE (*Ziegler's Beiträge*, 1896, 19, p. 433). Similar observations have been made in many other places (see WARTHIN, *loc. cit.*).

by v. Behring in his most recent contributions though (contrary to v. Baumgarten) on the basis of a postnatal infection.[1]

We will not here discuss the arguments for and against this hypothesis (see Latent Tubercle Bacilli in the Lymph Nodes) but proceed on the assumption that such latency in general scarcely takes place, and consider the cases of death from tuberculosis within the first three to four months which I have had opportunity to examine.

Case 1.—(Legal autopsy No. 14. 1898.) Child two months and six days old, supposed to have died from ill-treatment. It was learned that the father had consumption, was unable to work, and stayed at home to take care of the children while the mother went out to work. The mother was perfectly well (and was still so three years after the death of the child). She nursed the child when at home. The child was kept in a box or in a bureau drawer: the room was very dirty. The father was bedridden at times, coughed, and expectorated, supposedly in a tin can on the floor; he often had the child in bed with him. It was also stated that the father would chew pieces of bread and give the child. The child died suddenly. The father died of tuberculosis about one and one-half years later.

Autopsy: Length of body, 58 cm.; weight, 4,830 grams. The tonsils were not enlarged, the cervical nodes slightly so. One node was examined microscopically, and no tubercles found. The bronchial and hilus nodes were considerably enlarged and caseous (contained numerous tubercles). The lungs were studded, partly with scattered greyish yellow granules, partly with tubercles in groups, and contained also larger, firm, caseous, consolidated areas which showed beginning softening; microscopically ordinary tuberculous tissue and masses of tubercle bacilli. Here and there tubercles in the liver and kidneys; none in the mesenteric nodes.

The respiratory apparatus must here be considered primarily affected (and probably first the lymph nodes). That the infection occurred after birth must be taken for granted from what has been stated of the father's consumption, etc.

Case 2.—A female child two and one-half months old, who was well until about a month after birth, when diarrhea with mucous and bloody stools set in. Nothing is stated about tuberculosis in the parents or other relatives; the child had never nursed, and was fed on milk.

Autopsy No. 150, 1900: Length, 56 cm.; weight, 2,580 grams. Numerous miliary tubercles in the lungs, especially the right; in the right upper lobe also an almond-sized caseous consolidated portion. The tubercles between the lobes and beneath the pleura were arranged in bead-like rows proceeding from the hilus of the lung. The bronchial and hilus nodes were enlarged to the size of beans, caseated; perforation into the bronchi could not be demon-

[1] See also Warthin's work (*loc. cit.*) where the same possibility is emphasized (point 8 in his conclusions).

strated. The cervical nodes were enlarged; in a few of them as well as in a few mesenteric nodes caseous foci were found. Tubercles were found in the spleen, liver, and some of Peyer's patches.

In this case of advanced tuberculosis the bronchial and hilus nodes seem to have been primarily infected, and from these the lungs, and secondarily the intestine, mesenteric (?) and cervical nodes. There is no information of the existence of tuberculosis in the parents; it is probable that the child was infected after birth; and there is nothing in the distribution of the tuberculous changes to speak against their development after birth.

Case 3.—(Autopsy No. 135, 1900.) Child 10 weeks old, whose mother had died of pulmonary tuberculosis seven weeks after the birth of the child. The mother also had syphilis and the child had congenital syphilis. When six weeks old the child entered the dermatological ward of the hospital; how it previously had been fed is not known. At the autopsy the lungs were found to contain numerous tubercles which in places had coalesced to form large caseous masses; the left lower lobe contained a caseous focus of the size of a hazel nut. The lymph nodes at the hilus were of the size of beans and caseous. There was also a right-sided tuberculous fibrinous pleuritis; tubercles were seen in the spleen and liver. No changes in the intestinal tract. Microscopically typical tubercles were found, and huge masses of tubercle bacilli.

In this case the possibility of intrauterine infection must be admitted, although the most important localizations do not point in that direction. But nothing speaks against an infection after birth, for which abundant opportunity had existed; if so the respiratory tract most likely was the atrium. (However, the cervical nodes, unfortunately, were not thoroughly examined.)

Case 4.—Autopsy of a child not quite two months old (born May 1, died June 30). Weight 2,250 grams. Beneath the pleura of the posterior surface of the lower lobe of the left lung a few tubercles of pin-head size were seen. In this lobe there also was a pea sized nodule filled with a greenish yellow thick material surrounded by a caseous zone and outside this by yellowish-white tubercles. In the right middle lobe was a similar nodule surrounded by small tubercles. The bronchial nodes were enlarged, caseous, and softened in their central portion. Several tubercles in the spleen, and small tuberculous ulcers in the ileum; the mesenteric nodes were considerably enlarged, softened in their central portion.

It was stated that the mother had pulmonary tuberculosis, and died 16 days after the birth of the child. The child was partly nursed by another healthy woman. A rash appeared when it was a couple of weeks old. It died of diarrhea.

Here the possibility of either intrauterine infection or infection from the mother after birth must be admitted. The distribution of the tuberculosis would fit either mode.

Case 5.—Child two months and 26 days old. The mother had pulmonary tuberculosis and kept the child with her more than a month before it was taken to the hospital. The child died of considerably advanced tuberculosis of the lymph nodes of the chest and of the lungs. There is nothing to speak against the assumption—in fact, to a certain degree it seems likely—that the child was infected from the mother after birth and died of this infection before reaching the age of three months.

Case 6.—Child three months and nine days old, who had stayed with its consumptive mother for two and one-half months, hence abundantly exposed to infection. It died of an extensive tuberculosis of the lymph nodes of the chest and abdomen; there were also considerable pulmonary tuberculosis and small tuberculous intestinal ulcers. Probably this child was infected by the mother after birth; at least the lesions found are easily compatible with this view. On the other hand, it is difficult to tell whether the infection took place through the respiratory or digestive tract.

Case 7.—The same reasoning is still more plausible in the case of a child four months old, who had been with its tuberculous mother over three months, and had died from tuberculosis of considerable extent of the thoracic and abdominal organs. Here also infection from the mother after birth, either through the respiratory or digestive tract, seems probable.

Case 8.—Child two months and two days old who was with its tuberculous mother for a month when the mother had to be taken to a hospital, where she died from pulmonary tuberculosis. The child suffered from enteritis, and steadily lost in flesh. At autopsy the bronchial nodes were much enlarged and tuberculous, the mesenteric nodes also enlarged, and there were miliary tubercles in all the organs. Here it cannot be decided whether the child was infected before or after birth; either possibility must be admitted.

One might also in this connection consider the cases in which tubercle bacilli were found in the lymph nodes of little children although these cases are much more uncertain. Thus, latent bacilli were demonstrated in the cervical nodes of children two months, three and one-half months, and four months old, and in the bronchial nodes of a child one month and 22 days old. The mother of the latter child was tuberculous; the infection might have been intrauterine, or more likely postpartum (from the mother) without tuberculous lesions yet having developed. In the case of the other three, and older children, postnatal origin is still more likely; nor were there any statements in these cases that the parents suffered from tuberculosis.

From the foregoing descriptions and remarks it will be seen that Case 1 in all probability must be regarded as an instance of infection from the father after birth.[1] In Case 2 nothing was known of the health of the parents. In the other six cases, on the other hand, the mother was tuberculous and the possibility of intrauterine infection present, but infection might also very well have taken place after birth. It may also be noted that in two cases the children were born with syphilis, a combination which is not rare; at least it appears as if children with inherited syphilis are comparatively prone to tuberculosis.

What does general experience teach as to children born of tuberculous mothers?—First it must be stated that tuberculous women not rarely abort or give birth to still-born infants in which tuberculous changes rarely have been demonstrated. The causes of the abortions and still-births seem to vary much: infection of the fetus in utero in rare cases; intoxication of the fetus from the mother on account of the latter's tuberculosis; poor condition of nutrition in the mother and thereby also of the fetus; and tuberculous changes in the placenta with their effect on the nutrition of the child must not be forgotten. It seems as if the latter changes are demonstrable comparatively often if only sufficiently painstaking examination is made. In this connection reference may be made to Warthin's work and to the recent investigations of Schmorl and Geifel.[2]

In this connection the following observations are of interest:

1) A woman 27 years old who for nine years had had symptoms of pulmonary tuberculosis, two months before death gave birth to an eight month's fetus (the latter unfortunately was not submitted for examination). The autopsy of the woman revealed chronic pulmonary and laryngeal tuberculosis, tuberculous peritonitis, left-sided salpingitis (of long standing) and a tuberculous endometritis; the placental site was uneven and nodular, and the whole inner surface of the uterus covered with yellowish white caseous masses which were especially abundant at the placental site. Microscopically, masses of degenerated tubercles, and tubercle bacilli. Here it is natural to suppose

[1] Infection from the father during conception through the sperm without infection of the mother must be considered so rare—if it occurs at all—as to be left out of consideration.

[2] *Munch. med. Wchnschr.* 1901, 51, p. 1676. They examined placentas from 20 tuberculous women and could demonstrate microscopically tuberculous changes in nine (*i. e.*, 45 per cent), both with advanced and beginning pulmonary tuberculosis. In three of these cases they could not demonstrate tubercles or tubercle bacilli in the fetus (inoculation of guinea pigs was not employed).

that the tuberculous endometritis antedated and brought on the premature delivery; but an infection of the uterus from the tubes after childbirth cannot be excluded.

2) July 5, 1904 a six month's fetus was examined born of a mother with advanced pulmonary tuberculosis of about six months' standing. The child lived five hours after birth, was a little over 30 c.m. long and weighed 620 grams. Nothing worthy of note was found in its internal organs; a guinea pig was inoculated with pieces of liver and spleen; it was killed September 18 and no changes were found. The placenta weighed 180 grams and measured 10x10 c.m.; in its marginal portion several large infarcts were seen; otherwise no change. Two guinea pigs were inoculated July 5 with pieces of placenta; they became emaciated and when killed on September 18 showed signs of advanced tuberculosis proceeding from the point of inoculation. Microscopic examination failed to demonstrate tubercles or tubercle bacilli. The case will be further investigated.

However, it has been shown that most children born dead or alive of tuberculous mothers show no signs of tuberculous infection.

In this connection it is noteworthy that in the last six years 261 infants still-born or dead a few days after birth were examined at the pathological institute. Not a few were born of tuberculous mothers. Tuberculous changes were not demonstrable in a single case.

It must also be mentioned that among children which had lived for some time many were born of tuberculous mothers but were themselves without any trace of tuberculosis. The following cases will serve as examples:

1) Autopsy 70, 1902. Child, age 10 months; mother had pulmonary tuberculosis. The child died of bronchopneumonia. The cervical nodes were considerably enlarged, light greyish red in color. Nodes from both sides of the neck were inoculated into two guinea pigs, one of which died 21 days later (of enteritis), the other was killed after four months. Both animals were entirely free from tuberculosis. Both tonsils and three cervical nodes were examined microscopically, with negative result. The mesenteric and bronchial nodes of the child were very slightly enlarged, with no sign of tuberculosis.

2) Autopsy 128, 1902. Boy 11 months old whose mother died of pulmonary tuberculosis when the child was nine months old. It is stated that the mother at times masticated food for the child. The child died of enteritis, rickets, and bronchopneumonia. The cervical lymph nodes were swollen, soft, greyish red; two guinea pigs were inoculated with material from these nodes on July 19; they were killed November 7 and were in every respect normal. The lymph nodes at the root of the lungs were of the size of beans, the mesentric nodes were also enlarged, and pale. Microscopic examination of the various lymph nodes, tonsils, and spleen revealed no sign of tuberculosis.

3) Autopsy 45, 1903. A boy three months and 20 days old whose mother died of pulmonary and laryngeal tuberculosis two days after the birth of the child. The child was much emaciated, with the clinical findings of chronic staphylococcus pyemia. The autopsy revealed pale pea-sized cervical nodes, swollen axillary nodes, mesenteric nodes enlarged to the size of almonds (tracheal and bronchial nodes not enlarged), and enlarged spleen. March 12, two guinea pigs were inoculated with cervical nodes, one with axillary nodes, one with mesenteric nodes, one with spleen, and one with liver tissue; all animals were killed May 7, and no trace of tuberculosis was found in any of them. The cervical and mesenteric nodes, the spleen, and the liver were found normal on microscopic examination, and tubercle bacilli were not found in them.

4) Autopsy held November 7, 1903, on a child four weeks old, born seven weeks before full term. The mother had pulmonary and laryngeal tuberculosis and died 13 days after the birth of the child (father healthy). The child was bottle-fed, and brought to the hospital after the mother's death suffering from enteritis from which it died. The cervical and mesenteric nodes were slightly swollen, the tracheal nodes not enlarged. On November 7, four guinea pigs were inoculated with cervical nodes, mesenteric nodes, liver, and spleen respectively; all were killed December 22 and no tuberculosis detected. Microscopic examination of the tonsils and a group of mesenteric nodes gave negative results.

5) Autopsy 37, 1904. Child two months and five days old, born 10 weeks prematurely of a tuberculous mother. It was brought to the hospital soon after birth and died from marasmus and bronchopneumonia. The cervical and mesenteric nodes were enlarged, soft, light red. Two guinea pigs were inoculated March 5 with cervical and mesenteric nodes; they were killed May 3 and found to be perfectly healthy. Ten mesenteric nodes, eight cervical nodes, liver, spleen, and kidneys were examined microscopically with entirely negative result.[1]

The following observations may also be included here; the last one is also of medicolegal interest.

6) April 26, 1904, I received a six months' fetus born of a tuberculous mother (whose pulmonary tuberculosis had mainly developed in the last seven months). The placenta was not received. The autopsy of the fetus presented nothing of special interest; there was no sign of tuberculosis. One guinea pig was inoculated with pieces of spleen and mesenteric nodes, another with liver; both were killed two months later and found healthy. Microscopic examination of liver, spleen, and lungs failed to show tuberculous lesions or tubercle bacilli.

7) A married woman a little over 20 years old was confined to bed with pulmonary tuberculosis for many months. She had not the faintest suspicion of being pregnant. After staying in bed for seven months she suddenly and entirely unexpectedly gave birth to a boy; 10 days later she died from pul-

[1] These observations are in accord with those made at other pathological institutes; for instance, see report of LUBARSCH (*Arbeiten aus der path.-anat. Abtheilung des Hygienisches Instituts zu Posen*, 1901, pp. 7–17.

monary tuberculosis. The child was extremely emaciated, weighed 2,400 grams. It remained well for two years when it became sick first with gastro-enteritis, then cough, and died two years and three months old. The post-mortem showed advanced tuberculosis in the digestive tract, the lungs, and the brain. The possibility of a congenital tuberculous infection may not be excluded.

All these observations like similar ones published elsewhere speak against frequent intrauterine transmission of tubercle bacilli. Whether intrauterine transmission is sufficiently rare to be left out of consideration practically (as claimed by Robert Koch) is another question. With the cases related in mind I must lay somewhat more stress on intrauterine infection than is generally done, and declare myself in accord with Warthin and Cowie[1] in this matter.

Here another question suggests itself: Is it possible from the extent of the tuberculous lesions (in children dead of tuberculosis within the first months) to conclude with any certainty whether the infection took place before or after birth? This leads up to still another question which hitherto has received little attention: Can tubercle bacilli transmitted to the child in utero remain latent in the organs, for instance, the lymph nodes, later to proliferate and cause active tuberculosis (see v. Baumgarten's theory). Warthin and Cowie also touch on this question as they state in their conclusion (8) that "a true latent congenital tuberculosis is both possible and probable." Schmorl and Geipel[2] express themselves as opposed to the assumption of such a prolonged latency. They believe that tubercle bacilli not rarely pass from a tuberculous mother to the fetus; if few in number they soon perish; if more numerous they will produce in the fetus a tuberculosis which will cause death in early childhood.

Material with which to furnish proof in favor of prolonged latency of tubercle bacilli transmitted in utero is still very scanty. Thus it seems probable in cases of successful inoculations from blood or organs of new-born infants without tuberculous lesions that the transmission of tubercle bacilli took place during or shortly before birth. Still, the possibility of prolonged latency of the bacilli after an eventual intrauterine infection is to a

[1] *Loc. cit.* [2] *Loc. cit.*

certain extent supported by the fact previously stated that lymph nodes of children of various age have contained latent tubercle bacilli demonstrable by animal inoculations.[1]

The theory of congenital latency of tubercle bacilli, however, is far from being proved. It seems to be true in general that the body of the new-born is a favorable soil and that infection with tubercle bacilli is rapidly followed by active tuberculous inflammation.

How early may one suppose that a child infected with tubercle bacilli immediately after birth dies of tuberculosis? It is of course out of the question to give definite figures in days, weeks, or months. Many factors are brought into play: The quantity of infective agent, its virulence, and the power of resistance in the body. The point here is to determine the shortest time after birth in which death from tuberculosis can occur when an intrauterine infection may be excluded. Hence we can only use cases in which the mother was healthy. Our Case 7 seems to be one in point, as the mother is still living and healthy, while it seems that the father infected the child after birth. This child died at the age of two months and six days.

In the literature similar cases may be found. Thus Kossel[2] and Wassermann[3] describe a child 10 weeks old which died of tuberculosis. They thought it had been infected between the ninth and 17th day after birth, during which time it was kept in a room in which there was a large quantity of tuberculous sputum. The mother was healthy. Hochsinger[4] describes three cases of fatal tuberculosis in infants. The two infants whose mothers were tuberculous died at the ages of 31 days and 16 weeks. Here the possibility of intrauterine infection was present. The third infant died at the age of 38 days, and the mother is said to have been free from tuberculosis (nothing is said about the father). In this case infection after birth seems most probable.

Haushalter[5] also has described a child two months old with advanced tuberculosis, whose mother was healthy, while the father had tuberculosis. It was thought the child had been infected after birth.

From this experience it appears safe to state that a child infected with tubercle bacilli after birth may die from tuberculosis within two months.

[1] It is of interest to bear in mind that congenital tuberculosis is quite frequent in certain animals, especially calves; on the other hand I have no knowledge of investigations into the occurrence of latent tubercle bacilli in calves.

[2] *Ztschr. f. Hyg. u. Infektionskrankh.*, 1896, 21, p. 59.

[3] *Münch. med. Wchnschr.*, 1894, 41, p. 713.

[4] Cit. by DÜRCK in LUBARSCH-OSTERTAGS *Ergebn.*, 1895, 2, p. 196. [5] Cit. by DÜRCK, *loc. cit.*

Antvord[1] seems to place this age limit too high, when he states that not more than three or four months need elapse between the primary infection and death from tuberculosis. He also maintains that such cases are rare in which infants from six to seven weeks old die from so advanced tuberculosis that intrauterine infection must be assumed.

Cornet,[2] on the other hand, seems to place the limit rather low, when hestates that tuberculosis scarcely occurs in the first three or four weeks, "that is, not until the disease can be explained by a postnatal infection."

From the available facts it seems to me unlikely that a child infected after birth should die of tuberculosis when only from three to four weeks old. (It might be possible if it also had inherited syphilis, which perhaps promotes the development of tuberculosis.) But at any rate one may suppose that a child may die of postnatal tuberculous infection when eight weeks old, and probably even when only five to six weeks old.

THE MAIN LOCALIZATION AND POINTS OF INVASION OF TUBUCULOSIS IN CHILDHOOD.

The primary localizations of tuberculosis is now one of the burning questions, especially since Robert Koch's notable address before the Congress in London in 1901, in which he maintained that man is not infected with bovine tubercle bacilli, which are of another species than the bacilli in man, and stated as proof, that primary infection of the intestinal tract is a great rarity. The proof that this difference in species does not exist may be considered established by the numerous experimental investigations made, especially in Germany, England, Denmark, etc.

Concerning the rarity of primary localization in the intestinal tract considerable material previously existed, but too little attention had been directed to this localization. The older statistics also have been worked out from the one-sided point of view that infection as a rule takes place by inhalation and that the respiratory organs most frequently are primarily attacked. Interest in these questions was aroused by Koch's address, and still more so when v. Behring in 1903 advanced his hypothesis—so diametrically opposed to that of Koch—that infection most frequently

[1] *Norsk Mag. f. Laegev.*, 1895, 55, p. 1013. [2] *Loc. cit.*

takes place through the intestinal tract and already in infancy (but *after* birth as had also been maintained by Koch.)

Before relating my own investigations on this subject it may be of interest to consider the more important contributions to it in the literature. It must at once be noted that these contributions vary greatly in their scope and in their value. Some of them have been written from a clinical point of view, while a larger number are based on autopsy material, as a rule from long periods, most of the autopsies generally having been performed by others than the reviewer of the entire material. Many of these reviews are very one-sided, particularly because often the examinations had been made with the view to demonstrate the largest possible number of primary changes in a certain system of organs (e. g., in the intestinal tract in the English statistics.)

It is a constant feature that the mouth, throat, and their regional lymph nodes scarcely have received attention, except in the statistics of surgeons and pediatricians, where these very localizations dominate completely because they are the parts most readily examined in the living subject. Some statistics are only concerned with deaths from tuberculosis in children; others only with latent tuberculosis as it is found, for example, in children dead of acute diseases, such as diphtheria, etc. (Geill; Heller; Councilman, Mallory, and Pierce, etc). Nor do all these deal with children of the same periods of age. Some only consider children under five years, others under 10 or 12 (especially the English), others under 15 (which is also our limit), and again others simply throw adults and children together. The nationality of the author also seems to play a certain part. The English authors, at least, have results which generally differ greatly from those of the Germans (though the results have been more even in the last couple of years). However, the material has constantly grown larger; it has been treated from more numerous points of view; and hence has become more valuable.

We shall only note some of the more important contributions, especially recent ones and those from the Scandinavian countries.

Froebelius, whose extensive work already has been mentioned, also considers the localizations of tuberculous lesions. He believes the disease most

frequently originates from the bronchial nodes, in some cases from the mesenteric nodes, and that inhalation tuberculosis entirely outranks the alimentary form. He scarcely pays attention to congenital forms, though in 20 of his cases death occurred in the first two months (for instance, one child three days old; one, one week; one, two weeks; three, three and one-half weeks, etc.). In later childhood, however, both tuberculosis of the bronchial nodes and miliary tuberculosis decrease in frequency while that of the intestines and mesenteric nodes increases. Froebelius also points out that pulmonary tuberculosis in infants often appears in the trail of intestinal and pulmonary affections.

Geill, in his extensive work[1] (from Denmark), is mainly concerned with lymphatic tuberculosis. In 288 cases of latent tuberculosis in children the bronchial nodes were involved 262 times; cervical, 39; lungs and pleurae, 125; mesenteric nodes, 59; spleen, 47; and the liver 38 times. In 142 cases the lymph nodes only were attacked, namely the bronchial nodes 109 times; mesenteric, 22 times; bronchial and mesenteric, seven times; bronchial, cervical, and mesenteric, three times; bronchial and cervical, once. In 22 cases the tuberculous process was considered arrested and in 40 cases almost completely so. Of the 288 cases (from 902 autopsies), (1) the mesenteric nodes alone were affected in 23; (2) both the abdominal and thoracic organs in 77 cases; possibly 11 of these cases must be added to the first group as probable primary intestinal infections the number of cases of which then would be 34, or 11.8 per cent of all tuberculous cases. (3) The thoracic organs were affected in 188 cases (in 78 cases the bronchial nodes and lungs together, the former probably primarily), sometimes also the cervical nodes; in 109 cases the bronchial nodes (in one case also the cervical nodes). To this group must also be added 66 cases of the second group as of probable thoracic origin, which gives a total of 254 cases or 88.2 per cent of all tuberculous cases. Of the lymph nodes in the chest the hilus nodes were oftenest attacked; then those at the bifurcation of the trachea; in these groups the changes were marked. When the cervical nodes were involved Geill believed them to have been secondarily infected by an ascending tuberculosis. He concludes that inhalation tuberculosis is the most frequent form in children as well as in adults.

Müller,[2] in 150 cases of fatal tuberculosis found the lungs involved in 139 cases; the lymph nodes in 126; and of the latter the bronchial nodes in 103 cases; the mesenteric nodes in 72, etc. In the 59 latent cases the lymph nodes were involved in 44 cases, (bronchial, 27; cervical, six;[3] mesenteric, six; mediastinal, two etc.), the lungs in 34 cases etc. Müller believes that the intestinal tract, on the whole, is a rare point of invasion, and he points out that the mesenteric nodes are much more rarely primarily attacked than the bronchial. In Hecker's[4] 97 fatal cases the lymph nodes were affected in 90 cases: bronchial and mediastinal, 63; mesenteric, 26; cervical, 12; retroperitoneal, nine; the lungs were involved in 74 cases; the intestines in 22,

[1] *Loc. cit.* [2] *Loc. cit.*

[3] However he believes that this figure is too low as these nodes had not been sufficiently examined.

[4] *Loc. cit.*

etc. In the 50 latent cases the localization was in the bronchial nodes in 38 cases; mesenteric, five; cervical, three; also frequently in the lungs but as a rule secondarily from the bronchial nodes.

Boltz,[1] in his 176 fatal cases of tuberculosis in children, found the respiratory organs attacked in 131 cases: involvement of the bronchial nodes was absent in three only. In 72 cases (41.3 per cent) the digestive organs were affected, how often together with the lungs is not stated.

The result of the study of the frequency of the various primary localizations of tuberculosis by English authors are quite remarkable. Thus Woodhead[2] in 127 carefully examined cases of tuberculosis in children found tuberculous ulcers in the intestines in 43 cases (it is not plainly stated whether the respiratory organs also were affected). The mesenteric nodes were tuberculous in 100 cases; and affected alone in 14 of these (or in 11 per cent of the autopsies, especially at the age between one and five and one-half years; together with the hilus and mediastinal nodes in 69 cases, with the lungs in 62 (most frequently recent tuberculosis); and associated with tuberculous enteritis in 18 cases. In 27 cases (about 21 per cent) the hilus and mediastinal nodes were involved when the mesenteric nodes had escaped.

Still,[3] in his 269 cases of tuberculosis in children found the lungs attacked in 210 cases, the intestines and peritoneum in 141 cases. However, in 46 cases the point of primary invasion was difficult to locate. The lymph nodes give the best starting point to determine this. He came to the conclusion that the lungs were primarily attacked in 138 cases (105 certain, 33 probable), of these seven were under six months; 18, 6–12 months; 38, one to two years; 20, two to three years; 20, three to four years; 12, four to five years; nine, five to six years, etc.; the intestinal tract was primarily involved in 63 cases, or 23.5 per cent (53 certain cases, 10 probable); five of these were 6–12 months old; 15, one to two years; six, two to three years; 11, three to four years, etc. Infection by way of the lungs was most frequent; after the age of five years, however, equally frequent by the intestinal tract. It is also noteworthy that the ears, according to Still's investigations, were the point of invasion in 15 cases or seven per cent (especially in the first two years), the fauces only twice. Of 43 cases of latent tuberculosis the lungs were affected in 26, the intestines in 16 and the ears alone in one case (the mesenteric nodes alone in nine cases.)

Carr's[4] findings in autopsies on 120 tuberculous children agree with those of Still. He found the starting-point to be in the chest in 79 cases, in the abdomen in 20 cases or 16.6 per cent (in six cases a decision was impossible). In 26 cases with little advanced or ancient tuberculosis, the chest alone was attacked in 12, the abdomen alone in seven, the mesenteric nodes alone in five cases. In 53 cases under two years the disease was found to have commenced in the chest 43 times, in the abdomen five times; in 27 over five years 12 times in the chest and six times in the abdomen.

[1] *Loc. cit.*

[2] *Report from the Laboratory of the Royal College of Physicians*, Edinburgh, 1889. Vol. I.

[3] *Brit. Med. Jour.*, Aug. 19, 1899, 2, p. 454.

[4] *Brit. Med. Jour.*, Sept. 2, 1899, 2, pp. 627, 814.

Shennan,[1] collected 355 cases of tuberculosis in children from an Edinburgh hospital. He succeeded in determining the primary focus in 331 cases and 93 of these were cases of alimentary tuberculosis; in a later series of 45 cases, 11 were abdominal; in all 104 abdominal cases in 376, or 27.8 per cent. Carr cites investigations of Guthrie[2] according to which tuberculosis in childhood by far most frequently starts from the chest.

Still, Carr, and Shennan pay little attention to the organs of the neck. On the other hand, Walsham[3] mentions investigations by Batten (from St. Bartholomew Hospital in 1895) according to which in 100 tuberculous children the cervical nodes were attacked in 14 cases; the mesenteric nodes were tuberculous in 63 cases, and in 54 of these the nodes in the chest were also involved. Still and Carr, while giving high figures for the number of cases of abdominal tuberculosis, still maintain that the organs of the chest and the common atrium are most frequently primarily attacked. Raw,[4] however, gives most surprising figures, claiming to have observed 273 cases of tabes mesenterica. In 38 of these he performed autopsies when the lungs often were entirely unaffected (however details are lacking). Conditions are different according to the figures from America given by Hand[5] concerning 115 cases of tuberculosis. The lungs were apparently primarily involved in 75 cases (65.2 per cent), the mesenteric nodes in 10 (8.7 per cent), the tonsils in one case, primary focus not determinable in 29. The bronchial nodes were involved in 94 cases, the lungs in 70, the mesenteric nodes in 53 cases, the intestines in 28 etc. Most of the deaths occurred in early childhood, 60 under two years: 25, two to five years: 30, 5 to 12 years. Hand supposes that most cases are due to air infection, while in some cases infection is through food and by way of the intestinal tract. Moreover, anatomically these cases cannot be separated (as shown by Ravenel's experiment of feeding tuberculous material to monkeys followed by the development of primary tuberculosis of the thoracic organs), or at least with difficulty. This is contrary to Still who directly determined the point of invasion from the group of lymph nodes most affected.

Among other American observers Northrup[6] in 125 cases found the starting point in the alimentary tract three times. Bovaird[7] in 75 cases from a New York infant asylum found the bronchial nodes involved in all. Holt,[8] in 119 autopsies from infant asylums in New York found no case of probable primary gastrointestinal localization (although the mesenteric nodes were involved in 35 per cent of the cases and the intestine in 37 per cent).

Heinrich Schmidt[9] in 336 children under 10 years dead from tuberculosis concluded that the disease was primary in the respiratory tract in 230 (68.5 per cent), in the lymph nodes in 63 cases or 18.7 per cent (bronchial 32, all groups 19, mesenteric six, other groups 12.

[1] Cited by PRICE-JONES (*vide infra*). [2] Cited by PRICE-JONES. [3] *Loc cit.*

[4] *Brit. Med. Jour.*, August 29, 1903, 2, p. 470.

[5] *Proc. Path. Soc. of Philadelphia*, 1903, n. s. 6, p. 132.

[6] *N. Y. Med. Jour.*, 1891, 53, p. 201, cited after PRICE-JONES.

[7] *N. Y. Med. Jour.*, 1899, 70, p. 1, cited after PRICE-JONES.

[8] *Med. News*, 1896, 69, p. 656, cited after PRICE-JONES. [9] *Loc. cit.*

Racynskyi believes that infection in childhood most frequently takes place through the organs of respiration, as he found the bronchial and mediastinal nodes involved in nearly all fatal tuberculous cases, only 17 cases being exempt. He admits, however, that infection may take place by way of the digestive tract.

Monrad[1] also considers the respiratory tract the chief atrium, having found primary localization in the lungs and bronchial nodes in 152 cases and in the digestive tract in only five of his 157 fatal cases.

The figures of Fibiger and C. O. Jensen,[2] however, are different. In 213 autopsies from two Copenhagen hospitals tuberculosis existed as the cause of death, or as a complication, in 116 cases. There were 13 cases of primary affection of the intestinal tract and abdominal lymph nodes and two other cases probably of similar origin, that is, six (or seven) per cent. of all autopsies, or 11 (or 13) per cent of all tuberculous cases; 79 of the autopsies were in children, 25 of whom had tuberculous changes, and in four (or five) cases (16 or 20 per cent) these were primary in the digestive tract. These results, as we see, resemble those of the English observers.

Baginsky's[3] results are different. In his first series of 933 cases there was none of primary intestinal tuberculosis; in a later series of 806 cases, 144 (17 per cent) of which were tuberculous, there were six (4.1 per cent) of primary intestinal tuberculosis (compare the findings of Heller and Councilman whose percentages of tuberculous cases are about the same, but with much greater proportion of the intestinal form).

In Orth's[4] 203 autopsies in children over three months of age there were only two cases of primary intestinal tuberculosis in 47 tuberculous cases, that is one per cent. of all autopsies, and 4.25 per cent of the autopsies in tuberculous cases.

Nebelthau[5] directed his attention particularly to primary infection of the intestinal tract. In 26 autopsies on tuberculous children (from the Halle Polyclinic in three years) he believes primary infection of the digestive tract occurred five times (19.2 per cent), of the respiratory tract nine times (34.6 per cent of the digestive and respiratory tracts 12 times (46.1 per cent). He believes the infection generally could be traced to man.

Ganghofner[6] investigated 973 autopsies in children dead of acute diseases and found latent tuberculosis in 253 (26 per cent), and primary infection of the intestinal tract in only five of these (one-half per cent of all cases or two per cent of the tuberculous cases).

V. Hansemann[7] among his 8,000 or 10,000 autopsies discovered only 25 cases of primary intestinal tuberculosis; nine of these were in children under 15 years.

In the small but valuable statistics of Councilman, Mallory, and Pierce[8] of 220 children dead of diphtheria with 35 cases of latent tuberculosis (16 per cent) the intestinal tract was primarily infected in 13 cases (5.9 per cent of all cases or 37.1 per cent of the talent tuberculous cases). On the other

[1] *Hosp.-Tid.* 1902, R. 10, p. 300.
[2] *Hosp.-Tid.*, 1902, 4. R. 10, p. 923.
[3] *Loc. cit.*
[4] *Loc. cit.*
[5] *Loc. cit.*
[6] *Arch. f. Kinderheilk.*, cited by HOF and HELLER.
[7] *Berl. klin. Wchnschr.*, 1903, 40, p. 141; 170.
[8] *Loc. cit.*

hand, Morf,[1] who systematically examined the abdominal organs in 232 adult bodies (in 86, or 37 per cent of which there was tuberculosis), found tuberculosis of the abdominal lymph nodes in only two cases where tuberculosis could not be macroscopically demonstrated elsewhere.

Besides the English observers Heller of Kiel and his pupils have thoroughly traced the routes of infection, with special reference to the intestinal tract, and have energetically advocated the frequency of this route. Heller,[2] as the result of a study of the autopsy findings in 714 fatal diphtheria cases in children occurring between the years 1873 and 1894, states that in 140 cases (19.6 per cent) tuberculosis existed as an accidental finding, and in two of these (1.43 per cent) as primary intestinal tuberculosis, in eight cases (5.7 per cent) intestinal and mesenteric, and in 33 cases (23.5 per cent) as tuberculosis of the mesenteric nodes. To these must be added 10 cases (7.1 per cent) with involvement of the mesenteric nodes plus other organs than the lungs, making a total of 53 cases (37.8 per cent) of probable primary intestinal infection, or about one-third of all the latent cases. Heller ascribes the infection to milk from tuberculosis cattle.

Wagner's[3] publication is a continuation of Heller's work. Among the first 600 autopsies held at the Kiel Pathological Institute in 1903, 76 were in children, and in 13 of these (17.1 per cent) there was primary intestinal tuberculosis. He found altogether 28 cases of primary infection through the intestine, namely:

1) Eight cases of tuberculosis of the intestine and mesenteric nodes (all children under 15).

2) Seven cases of primary intestinal tuberculosis with dissemination to other organs (five children).

3) Thirteen other cases where tuberculosis no longer could be demonstrated but where the changes could only be interpreted as due to previous tuberculosis (e. g., calcified mesenteric nodes); three of these were in children, making a total of 16 children of the 76 (21.1 per cent) with primary tuberculosis of the intestinal tract.

Hof[4] has systematically gone through the 15,000 autopsy records of the Kiel institute in the last 30 years to investigate the frequency of primary intestinal tuberculosis. Of 7,683 autopsies in adults tuberculosis was found in 2,697 (35.1 per cent), and of 4,649 in children in 936 (20.1 per cent). In 408 of the latter cases (43.5 per cent) the lungs and intestinal tract were affected, and in 80 of these he considered the intestine to be primarily affected. Adding the cases of undoubted primary intestinal infection we get a total of 235 or 25.1 per cent of all autopsies in children, and primary tuberculosis of the respiratory tract in 527 or 56.2 per cent. Of the 2,697 adult cases 159 (5.9 per cent) were primary in the intestinal tract, while in 84.9 per cent the respiratory tract was primarily involved. Heller, in his most recent article,[5] adds that in 230 later autopsies, 23 of which were in children, he found recent or

[1] *Trans. Chicago Path. Soc.*, 1903, 5, p. 245.

[2] *Deutsche med. Wchnschr.*, 1902, 28, p. 696.

[3] *Münch. Med. Wchnchr.* 1903, 50, p. 2036; 2095.

[4] *Ueber primäre Darmtuberkulose nach 15,000 Sectionen.* Inaugural Dissertation, 1903.

[5] *Berl. klin. Wchnschr.* 1904, 41, p. 517.

old intestinal tuberculosis in 12 per cent of the adults and in 26 per cent of the children.

From the recent statistics of 1,820 autopsies collected by Lubarsch[1] we learn that tuberculous changes were found in 60.6 per cent of all cases, and in 69.1 per cent of those over 16 years of age. In the 297 children tuberculosis was found 63 times; 14 of these cases were instances of primary alimentary tuberculosis 21.2 per cent (or 4.7 per cent of all children). Of 52 children over one year 12 or 23.8 per cent had primary alimentary tuberculosis. Among all the 1,087 cases of tuberculosis there were 56 (5.15 per cent) of the alimentary type. By including cases of coincident infection of the mesenteric and bronchial nodes and of primary tonsillar tuberculosis Lubarsch puts 30.8 per cent as the greatest possible proportion of infection through the digestive tract.

Hof[2] gives the following summary:

OBSERVER	NUMBER OF TUBERCULOUS CHILDREN	PRIMARY IN DIGESTIVE TRACT		PRIMARY IN RESPIRATORY TRACT		BOTH RESPIRATORY ARD DIGESTIVE TRACTS AFFECTED	
		Cases	Per Cent.	Cases	Per Cent.	Cases	Per Cent.
Carr	105	20	19.0	79	75.0	6	5.7
Still	247	63	25.5	138	55.9	46	19.0
Kossel	22	1	4.5	13	59.0	8	36.4
Nebelthau	26	5	19.2	9	34.6	12	46.0
Hof	936	235	25.1	527	56.2	103	11.0

Primary tuberculosis of the mesenteric nodes alone was found by Hof in 67 cases or 7.2 per cent; Woodhead in 14 of 127 cases or 11 per cent; Carr in five of 120 or 4.2 per cent; Still in nine of 269 or 3.3 per cent; Bouvaird in three of 200 or 1.5 per cent.

To illustrate the difference in the statistics from different countries Bouvaird gives the following table:

SOURCE	NUMBER OF CASES	PRIMARY INTESTINAL TUBERCULOSIS	
		Cases	Percentage
Germany	236	9	4
France	128	..	..
England	748	136	18
United States	369	5	1

Bouvaird has used most of the statistical data mentioned here but can hardly have included those of Heller and his pupils.

Among the most recent English contributions is that of Symes and Fisher,[3] based on autopsy material from hospitals in Bristol. Among 500 fatal tuberculous cases 102 were in children under 12 years. In 12 of these (11.7 per

[1] *Loc. cit.* [2] *Loc. cit.* [3] *Brit. Med. Jour.* 1904, 1, p. 884.

cent) the abdominal organs were primarily attacked, in 57 (55.8 per cent) the respiratory organs. Of the remaining cases, in four probably the bones and joints were primarily attacked, in four the skin, in one the tonsils, and in four no primary focus was demonstrable. In children under two years the ratio between primary abdominal forms and primary respiratory forms was as five to nine. The following table gives the ratio at various ages:

Age	Ratio
0–12 years	1 : 4.7
10–24 years	1 : 3.9
25–36 years	1 : 9.7
37–48 years	1 : 9.25
49–60 years	No primary abdominal case

Kingsford[1] gives details of 339 children under 14 who died of tuberculosis. One hundred and sixty-two (or 48 per cent) had died in the first two years and 270 (or 80 per cent) in the first five years. Two hundred and twelve (62.5 per cent) were considered infected through the respiratory tract; 64 (18.9 per cent) through the digestive tract: 13 (3.8 per cent) through nasopharynx or middle ear;[2] in 50 cases (14.7 per cent) the primary focus was doubtful. He believes it possible to exclude congenital tuberculosis. He admits, in regard to the first group, that the bronchial nodes, which were most frequently attacked, may be infected from the throat or the intestinal tract. As to the second group, the mesenteric nodes were most frequently affected; infection from food, especially milk, he considers unimportant. Kingsford also has summed up the statistics of various countries as to the frequency of intestinal tuberculosis: English authors (Still, Shennan, Guthrie, Carr, Ashly, Batten) record 214 cases of intestinal tuberculosis among 1,119 tuberculous cases, i. e., 19.1 per cent. If Kingsford's cases are added we have 278 out of 1,458 cases or 19.06 per cent.

American authors (Northrupp, Bouvaird, Holt) found 13 in 434 cases or three per cent. French and German authors nine in 364 or 2.5 per cent.

Price-Jones[3] held 55 autopsies in children and found tuberculosis in 21 cases. He chiefly endeavored to determine whether infection was due to inhaled bacilli or to bacilli swallowed with food, and to decide further, if possible, whether the bacilli came from human or animal sources: he found the latter source to be improbable. Six cases were found to be alimentary; one surely respiratory, and five probably so (including two primarily mastoidal (?); finally nine too extensive to permit of determining the original focus.

In Norwegian literature different opinions on this subject have been expressed.

Professor H. Heiberg,[4] who always devoted much attention to tuberlosis, placed pulmonary tuberculosis first; secondly, intestinal tuberculosis, especially in children; finally, the more local forms among which he gave tuberculosis of lymph nodes a comparatively unimportant place. During the

[1] *Lancet.* Sept. 24, 1904, 2, p. 889.

[2] Kingsford adds that he considers the figures for this group too small.

[3] *Practitioner.* 1903, 71, p. 191.

[4] *Die Tuberkulose in ihrer anatomischen Ausbreitung.* Leipzig, 1882.

lively discussions in 1896–97[1] Bugge and A. Holst advocated the predominating rôle of inhalation tuberculosis, while Malm, with equal energy, insisted on the importance of infection through the digestive tract with secondary localization in the thoracic organs, and Antvord emphasized the important part played by infection in childhood in the cases of tuberculosis which break out in adult life. In 1903–4[2] infection by way of the intestinal tract was again emphasized by K. Thue and Malm. A. Holst[3] demonstrated tubercle bacilli in various "scrofulous" affections, for instance, of the eyes (two cases, of 13 examined) and hypertrophic tonsils (five of six examined). J. Jensen[4] relates a clear-cut instance of probable alimentary tuberculosis. In an otherwise healthy family with nine or ten children one child three years old was taken with ill-defined intestinal symptoms and died of tuberculous meningitis. Some months later, another child, 16 years old, developed diarrhea; then tuberculous arthritis of the elbow; finally pulmonary tuberculosis, and died. They had only one cow whose unboiled milk they all drank; when the cow was killed it was found tuberculous throughout, including the udders.

Let us now turn to the study of my series with a view to determining the primary foci. Of the 39 cases of fatal (or in two cases of rapidly advancing) tuberculosis in Series I we may consider that 19 originated in the lungs and corresponding groups of lymph nodes, five originated in the digestive tract (namely, four in the intestines, generally associated with affection of the mesenteric nodes; one in the tonsils) 15 were of doubtful origin.

The latter 15 cases may be subdivided as follows: Six primary in the respiratory or digestive tract; five in the respiratory tract or osseous system; two were cases of general tuberculosis of lymph nodes; one was primary in skin, bone, or joint; one undetermined.

Of the 33 cases in Series II (1901–4) we may consider that 12 originated in the lungs with their groups of lymph nodes, four in the digestive tract (two in the intestinal tract, one in the cervical nodes, one in intestinal tract or cervical nodes), and 17 doubtful, namely: 13 primary in the respiratory or digestive tracts (seven probably from the intestine, six probably from the cervical nodes); three cases of general lymph node affections; one primary in cervical nodes or a joint.

The difference between the findings of these two series is seen to be that the doubtful cases are most numerous in the second

[1] *Norsk Mag. f. Laegev.*, 1896 and 1897, supplement.

[2] *Norsk Mag. f. Laegev.*, 1903 and 1904, supplement.

[3] *Klinisk Aarbog*, II. 1885, p. 193. [4] *Tidsskrift for den norske Laegeforening*, 1898.

series, in which the material had been most thoroughly worked up, and where infection by way of the throat seems to play a considerable part.

If we combine the two series we have 72 fatal cases (more accurately 69 fatal and three far advanced), in which the primary seat of the disease was located as follows:

Respiratory organs	31
Digestive organs	9
In 32 cases doubtful or in other organs, namely:	
Respiratory or digestive organs (eventually intestine, 13, cervical nodes six)	19
General lymph node tuberculosis	5
Respiratory or osseous systems	5
Throat or joint	1
Bone, joint, or skin	1
Doubtful (partial autopsy)	1

We must also add the 27 cases of latent tuberculosis, most frequently found in the lymph nodes, in which the disease was located as follows:

Intrathoracic lymph nodes only (sometimes also lungs)		16
Cervical nodes,	*i. e.*, digestive tract 2	1
Mesenteric nodes,		1
Cervical and intrathoracic nodes		3
Abdominal and intrathoracic nodes		1
General lymph node affection		5

It is also of great importance to include the 18 cases in which latent bacilli were demonstrated, namely in

Cervical nodes only,	*i.e.*, 15 in digestive tract only	13
Mesenteric nodes only,		1
Cervical and mesenteric nodes,		1
Intrathoracic nodes only		1
Cervical and tracheal nodes		1
All three large groups		1

The latter table alters materially the combined result. If we combine the fatal and all latent cases, $72 + 27 + 18 = 117$ cases, the distribution as to primary seat is as follows:

		Cases	Percentage
Primary in respiratory tract	(31+16+ 1)	48	41
Primary in digestive tract	(9+ 2+15)	26	22
Primary in respiratory or digestive tract	(19+ 4+ 1)	24	20.5
General lymph node tuberculosis	(5+ 5+ 1)	11	9.4
Doubtful, or other primary seats		8	6.8

In connection with these statistics it must first be noted that by primary seat in the respiratory tract usually is meant tuberculosis affecting the lungs or lymph nodes within the thorax (bronchial, hilus, and tracheal nodes). Under primary seat in the digestive tract is included not only tuberculosis of the intestinal tract and the lymph nodes connected with it but also primary tuberculosis of the tonsils and cervical nodes. While the former group of organs as a rule is thought to be infected by inhalation, the cause of primary tuberculosis of the digestive tract is first of all looked for in contaminated food, and hence it seems correct to include the oral cavity, especially the tonsils and the most important lymph nodes in the upper part of the neck.

In connection with tuberculosis of the latter organs (as also of the intestines) the question may always properly be asked whether in at least some of these cases the infection is not due to inhaled bacilli which have become mixed with the food. A separation in this respect, however, is impossible; but it does not seem likely that such a mode of infection should be frequent.[1]

By inoculation of calves to test the virulence of the tubercle bacilli facts might be gained which would aid in deciding in a given case of primary tuberculosis of the digestive tract whether the infection came from man or animals. But such investigations have not been made, and I have been compelled to pass this question by. However, I will here call attention to the fact to which I will return later, that in numerous cases of tuberculosis in children an existing tuberculosis among the surroundings, mainly in the parents, appears to be the most plausible source of infection.

We must critically consider more in detail the most important localizations of tuberculosis in children. We will commence with the respiratory organs. As seen from my tables of 117 cases, probably in over half of these the disease must be looked upon as primary in the respiratory tract (just how many of the 24 cases in group 3 to add to group 2 cannot be stated). And by comparing the other statistical data mentioned we find that the percentage of

[1] I quite agree with what Lubarsch in his last work (*loc. cit.*) states in regard to this question.

primary respiratory infections is steadily declining while that of digestive infections becomes relatively higher in recent statistics (percentages of respiratory infections are: Geill 88.2, Still 51.3, Carr 74, Hand 65, Schmidt 68.5, Symes and Fischer 55.8, Kingsford 62.5).

In this primary respiratory group we include infections of all groups of lymph nodes within the thorax. And the general rule in cases of children is that the lymph nodes are primarily attacked and that the lungs are infected from them. General experience teaches that isolated tuberculosis of bronchial nodes is quite common, while isolated pulmonary tuberculosis with or without a slight and plainly secondary lymph node tuberculosis is a rarity in children; such cases are oftenest met with in late childhood when pulmonary tuberculosis begins to present the features common in adults. As to the nodes in the chest, the common supposition, which also corresponds with the prevalent ideas regarding inhalation, that the bronchial nodes in a narrow sense are those first and most severely affected is not correct. On the contrary, one finds more frequently that the disease is in the hilus nodes, or in the nodes located along the trachea and about the bifurcation, which alone may be affected, or at least most affected. So the impression is created that the bronchial nodes proper are secondarily infected from them. Most frequently caseous foci are found in these nodes; comparatively rarely has microscopic examination revealed latent tuberculosis, or inoculations; latent bacilli. The latter fact is striking compared to the frequency of bacilli in the cervical nodes. It is partly, but not at all entirely, explained by the most extensive examination made of the cervical nodes. It appears that the cervical nodes are more important foci for the deposition of latent bacilli.

We will not at present enter into the way in which these nodes are infected. It lies most closely at hand to assume an air infection; but a descending infection from the cervical nodes or an ascending one from the abdominal nodes cannot be excluded. The predilection of the infection for the tracheal and hilus nodes might point in this direction.

The lungs, then, are regularly secondarily infected. As my

observations plainly show, this most frequently takes place in this manner: that the bronchial nodes rupture into a larger bronchus, generally near the hilus, and thus a secondary caseous bronchopneumonia is set up in the area of distribution of this bronchus. This is a very frequent occurrence (observed 14 times in our cases), and the secondary pulmonary affection is then found in the various lobes, as it appears, without predilection for any particular lobe, though a prevalent localization in the lower lobe is maintained by some. By subsequent breaking down of the caseous portions cavities arise, which are not at all rare in children (observed seven times in our last series). Sometimes cavities are partly due to compression of a bronchus with peripherally located bronchiectases. Nor is even fatal hemoptysis rare.

Such a mode of distribution will regularly lead to the development of progressive pulmonary tuberculosis, and further, often to a dissemination of the disease in the other internal organs where miliary tubercles, as a rule, are found, as in other cases of advanced tuberculosis of some internal organ. The easy and rapid dissemination of tuberculosis through lymph and blood channels is characteristic of tuberculosis in childhood.

However, the lungs may be infected from the nodes in other ways, such as by retrograde transport through the lymph channels. This lymphogenic infection is also quite frequent, though hardly as frequent as the other manner described. The invasion here takes place from the hilus and proceeds along the lymph channels and nodes which accompany the bronchi; the interalveolar connective tissue becomes involved, then an intraalveolar process is set up and finally we have a pulmonary tuberculosis with marked caseation and rapid extension.

Infection of lung tissue from lymph nodes in the chest or elsewhere may also take place by the blood stream; however, according to our present knowledge this route of infection does not appear to be as important as the other routes. We shall not at present enter into the possibility of infection from the intestinal tract after the intestinal mucosa has been passed and tubercle bacilli have entered the blood either directly or after having forced their way through a greater or smaller part of the lymph

vascular system. The occurrence of latent bacilli in mesenteric lymph nodes might point in this direction, and experiments on animals (rabbits, monkeys, etc.) in which, after feeding tuberculous material, only pulmonary tuberculosis was found, have established that this mode of infection is not of little importance. In our cases we most frequently found the lymph nodes of the chest affected together with the lungs, so it is most natural to look for the infection there.

Finally, we see that tuberculous processes, for instance in the lymph nodes, may extend to the lung tissue and infect it directly; however, this is of rare occurrence.

Compared with these secondary infections of the lung tissue from the lymph nodes a primary infection of the former is relatively rare, and as a rule only seen in older children. At the same time we then generally notice a change in the character of the disease, which tends to a more chronic course, to fibrous induration, and to apical localization. It is also in later childhood, after the seventh or 10th year, that the latent and obsolete cases are most commonly met.

In general, the older the tuberculous child, the less prominent the lymph node affections, and the greater the resemblance to the customary adult features as to localization, course, and dissemination. All transitions may occur. On the other hand, in young adults we may encounter features suggestive of pulmonary tuberculosis as seen in children, such as rupture of lymph nodes into bronchi, circumscribed caseous bronchopneumonias, etc. It must be added that with the cases of primary tuberculosis of the respiratory organs we also have included a few cases in which there also was some involvement of the digestive tract (tonsils, cervical nodes, intestines, or mesenteric nodes) but where this involvement was slight and plainly secondary in nature.

We will next consider cases of primary tuberculosis of the digestive tract which, it will be remembered, included infections through the throat as well as through the intestine, and which in our series numbered 26 cases, or 22 per cent. There were nine fatal cases of this class, in six of which the infection was primary in the intestine, while in three we must assume a simultaneous

infection of the intestine and throat. It is quite often necessary to assume primary infection in different localities at the same or different times, as has also recently been maintained by Ribbert and Lubarsch.[1]

In a few of these cases there was so extensive affection of the lymph nodes (also in the thorax) that one might consider other sources of infection of these nodes than the intestine; but the intestine, at any rate, must be considered primarily attacked, as the numerous extensive ulcers could in no manner be accounted for by the tuberculous nodes.

In some of these cases there were also small tuberculous foci in the lungs, but plainly of comparatively recent date, without softening (in one case only demonstrated microscopically), and they must be considered of secondary, perhaps hematogenous, origin; at any rate, they could not account for the infection of the intestinal tract. I, therefore, venture to maintain that in these cases there was a simultaneous infection of two or more parts of the digestive tract (intestine and throat). A few instances will be briefly stated; otherwise the reader is referred to the detailed publication, previously mentioned, where the whole casuistry is related.

1) Girl, 13½ years old, died of suppurative meningitis. Autopsy: Both tonsils and the cervical nodes enlarged, contain numerous tubercles. In the ileum and mesenteric nodes also groups of tubercles. The bronchial nodes were scarcely swollen. Miliary tubercles in the various internal organs.

2) Boy, 13½ months old, whose mother died of pulmonary tuberculosis when he was five or six months old. At the same time he developed a rash and swollen cervical nodes, especially on the right side, diminishing in size downward. The child gradually failed, and died of bronchopneumonia and diarrhea. Autopsy: Cervical nodes on the right side enlarged to the size of a pigeon's egg, caseous, in a continuous chain downward along the neck and into the posterior mediastinum; at the bifurcation and at the pulmonary

[1] Instances of separate infection of different groups of lymph nodes have often been observed, especially: 1) calcareous bronchial nodes and microscopic tubercles in the cervical nodes, and 2) caseous foci in the bronchial and hilus nodes, and in the mesenteric nodes. To relate a few instances: (*a*) In a seven-year-old child, dead of enteritis, there were calcified tracheal nodes and fresh tubercles, demonstrable microscopically, in a cervical node. (*b*) Child, 14 months old, which died of miliary tuberculosis; there were large caseous tracheal and hilus nodes, a few microscopic tubercles in one tonsil, and tuberculosis of a few cervical nodes; finally, numerous tubercles of microscopic size in the mesenteric and retroperitoneal nodes. Here it seems reasonable to suppose that the tonsils and cervical nodes, and perhaps the abdominal nodes, have been infected separately, and later than the nodes in the chest. But extension of the disease from the thorax to the other regions cannot be absolutely excluded.

hila the nodes again were increased in size, caseous, and continued outward along the bronchi. Tonsils normal. No pulmonary tuberculosis, but there were tuberculous ulcers in the intestines and numerous large caseous mesenteric and retroperitoneal nodes.

The clinical and post mortem findings are in favor of a primary infection of the digestive tract, both through the mucosa of the mouth and the intestine, and a descending lymphatic tuberculosis from the neck, and an ascending one from the intestine.

Can we now in these cases obtain data to prove how the infection took place whether by food, and then particularly by bacilli in milk of tuberculous cows, as maintained by Heller; or from human sources, by bacilli which in some way have gained access to the digestive tract, for instance, by contamination of food, or by first being inhaled and then swallowed? Many writers, as Volland, ascribe tuberculosis in children to uncleanliness, the bacilli reaching the oral cavity by means of soiled fingers (on which tubercle bacilli have been demonstrated).

I cannot go farther into this question, which has not been given detailed study. Thus I have not determined the virulence of the tubercle bacilli in the particular cases, as, for instance, might have been done by inoculations in calves (see the investigations of Fibiger and C. O. Jensen). I shall confine myself to pointing to the existence of tuberculosis in the parents or other near relatives in several of these cases, thus rendering an infection from human sources most probable. On perusal of the records in a series of tuberculous children it is, on the whole, striking how often the disease in little children must be brought in relation with disease among the surroundings, especially in the mother.

To the primary infections of the digestive tract we must further add two cases of latent tuberculosis of the cervical nodes and in the mesentery (due to double infection, occurring simultaneously or at different times), and also the 15 cases in which inoculation of guinea pigs revealed latent tubercle bacilli in lymph nodes (cervical, 13 times; mesenteric, once; both, once).

The importance of the latter findings has already been emphasized. Future control investigations are, of course, necessary; yet it may be stated that these findings strongly point toward the frequency of infection with tubercle bacilli through mouth

and pharynx, which also completely agrees with clinical experience regarding tuberculosis of the cervical nodes in children.

At present only hypothetical statements can be made as to whether this finding must be given a more far-reaching importance in the explanation of the origin of tuberculous infection of the internal organs in general—for instance, of the lungs. As these bacilli must have penetrated the mucous membrane of the throat somewhere, it may readily be conceived that they may extend farther along the lymphatics, or enter the blood and subsequently infect the different viscera. The same line of reasoning would hold for the latent bacilli which occur in other nodes, as the mesenteric.

It is peculiar how relatively rarely latent tuberculosis or latent tubercle bacilli have been demonstrated in the mesenteric nodes. Particularly when we bear in mind the observation of Heller and the English authors. This may partly be due to the relatively greater attention given to the cervical nodes. But sufficient microscopic examinations and inoculations of mesenteric nodes were made to incline me, at least at present, to ascribe less importance, so far as frequency is concerned, to infection through the intestinal mucosa than to that through the throat, providing we may draw conclusions as to the point of infection from the localization of the tuberculous process or of the latent bacilli. The figure of 22 per cent as representing primary infection through the intestinal tract is not as high as the figures reached by several recent statistics, but quite high when we bear in mind that the percentage of certain primary infection through the respiratory tract could not be placed at more than 41 per cent. In other words: Of the cases where the the primary localization can be determined with fair certainty, it is found to be in the respiratory tract twice as often as in the digestive tract, according to our statistics. It must, however, be remembered that in the 22 per cent we have included cases of infection of the tonsils and cervical nodes. (Compare Kingsford 3.8 per cent and Batten's 14 per cent of the latter class of cases). If only primary tuberculosis of the intestinal and mesenteric nodes had been included the percentage would have been much less. Our figure

is not suitable for comparison with other statistics of primary tuberculosis of the digestive tract as in most of these attention has only been given to the intestine and mesenteric nodes.

Only Lubarsch also included the tonsils (and also simultaneous infection of other organs) to reach his highest percentage of 30.8.

It is, on the whole, striking how little consideration has been given to infection through the throat and cervical nodes, even by those writers on tuberculosis in childhood who seem to emphasize infection through the alimentary tract. Statements are seldom made concerning it (as by Batten, Still, and Geill who, however, state that the nodes generally are secondarily attacked.) The reason seems very largely to lie in the fact that the organs of the neck and mouth and the upper cervical nodes are not sufficiently examined at autopsies, as well as in the scarcity of microscopic examination and inoculations of material from these nodes. The tonsils (including the pharyngeal tonsils) are an exception to this rule, as within recent years they have been the object of many systematic investigations. The percentages of infection (four to five) are not high, but if these cases were included and also the cervical nodes examined, I have no doubt, bearing my own observations in mind, that this route of infection would assume a relatively greater importance.

In connection with the two groups of primary infection through the respiratory and digestive tracts we have a third large group, where the tuberculous changes have their seat in both of these regions but where the extent of the changes does not permit a decision as to the primary seat.

Of the 24 cases in this class (20.5 per cent), those in which death was due to tuberculosis are naturally most difficult to judge as the changes here were greatest. In looking at the 19 cases of this kind it is seen that the question generally is of infection either of the respiratory or the intestinal tract (13 cases), the mouth and throat with their lymph nodes coming into consideration, much more rarely (six cases). In about two-thirds of these cases a primary infection of the respiratory tract must be assumed if we consider the extent of the pathological changes. One point must be emphasized: even when the thoracic organs are found

most affected at autopsy the clinical history may point either to early involvement of the cervical nodes and a descending infection of the throat, or to preceding intestinal infection of long duration.

To the cases of fatal tuberculosis four latent cases must be added, as in three cases the nodes in the chest were involved together with the cervical nodes, and in one case together with the mesenteric nodes; finally one case of latent tubercle bacilli in the tracheal and mesenteric nodes. Whether each of these groups was primarily infected, or one from the other, cannot be definitely determined. The former alternative appears most likely, especially on account of the finding of tubercle bacilli in the different nodes.

The small group of eight cases of tuberculosis of bones, joints etc., and also of lymph nodes (in the chest or neck) will not be given detailed attention. Evidently the lymph nodes here most frequently represent the primary focus from which the other organs were attacked, most likely by hematogenous infection.

On the other hand we must devote some further attention to the generalized tuberculous affections of lymph nodes. There were not less than 11 of these cases (9.4 per cent), five in cases of fatal tuberculosis (miliary tuberculosis, meningitis, etc.), five cases of latent generalized lymph node affections (as a rule caseation in the various groups; in one case only demonstrated microscopically, not by inoculation), and finally a case of latent tubercle bacilli in all the chief groups.

Here the question is raised: are the different groups infected separately, about simultaneously or at different times, and through their respective mucous membranes, or have we to deal with infection of only a single group from which the others are attacked so rapidly and violently as to give the appearance of a general infection?

That it may occur in the former manner we have already mentioned in discussing cases of advanced tuberculosis of both the respiratory and digestive tracts. This explanation is the more plausible in cases in which the changes are little advanced and located in lymph nodes which do not directly communicate. The

same is true of cases in which only latent bacilli in two or more groups are found.

In certain cases, however, we cannot deny the possibility of hematogenous infection of a large part of the lymphatic system. The lymph nodes seem to be a favorite location for the tuberculous virus also in cases of hematogenous infection. This is well known to be so in the certain cases of congenital tuberculosis in man and animals.[1] It is then natural to assume a similar mode of infection in certain cases, especially in the first months of extra-uterine life.

As often, and probably much oftener, the infection spreads from a certain primary focus to the other groups. The most frequent findings are the following: Swollen and generally caseous nodes beneath the lower jaw, and along the entire neck (generally decreasing in size downward), sometimes also in the supraclavicular and axillary regions and, further, in the posterior mediastinum along the trachea, in the pulmonary hilus, and outwards along the bronchi; finally in the mesentery and retroperitoneal tissue from the diaphragm downward along the spine and iliac vessels, and sometimes extending to the inguinal regions. Apparently the affection everywhere is equally advanced, equally old. Such an extension may take place in many ways. The one most commonly thought of is extension from the nodes within the chest in various directions, both because these nodes, according to general opinion, most frequently are found primarily attacked, and because not rarely the affection in this locality appears more marked and perhaps older than in the other localities. Accordingly, the nodes in the hilus of the lung, in the bifurcation of the trachea, and along the trachea most likely have been primarily infected, and from here the infection has spread outward along the bronchi, upward along the trachea to the neck (where, however, the nodes situated highest up very often are most affected),[2] finally downward in the retroperitoneal tissues, and from here to the

[1] The localization of congenital tuberculosis of man and animals is also remarkable. The lymph nodes especially affected are the periportal, retroperitoneal, and bronchial nodes.

[2] Naturally those cases must be left out of consideration where rupture into bronchi has taken place, in which the cervical nodes may be infected from the mucous membrane of the throat by tuberculous expectorate, which is known to take place regularly.

mesentery and along the iliac vessels, etc. Extension from the thoracic to the retroperitoneal nodes is often very plain and conspicuous. This mode of extension is, in fact, quite frequent. However, the point of origin may also be in the cervical nodes. In that case we generally find enlarged nodes along the entire neck, generally decreasing downward; then the supraclavicular and axillary nodes (very generally, as in surgical experience) are attacked, the process descending further to the posterior and anterior mediastinal and other thoracic groups, and finally to the retroperitoneal and other abdominal nodes.

Even where the entire clinical picture decidedly points to a descending cervical lymphatic tuberculosis and where the swelling decreases downward to the clavicle, one finds, as a rule, that it then again increases downward to the hilus of the lung. From this finding one might be inclined to deny the assumption that the thoracic nodes were secondarily involved and maintain that they either were infected separately (from the respiratory tract), or even that they were the starting point. But, in my opinion, this is scarcely correct. The whole evolution often points so decisively to a continuous process, and to extension from above downward, that an interpretation on that basis must be accepted. The marked swelling and pronounced cheesy degeneration of the hilus and tracheal nodes must then be explained by the consideration that these nodes are large, numerous, and perhaps also predisposed to marked swelling. Much seems to point in this direction; at any rate one hardly is justified in denying a continuity in the process even if there is an appreciable diminution in the size of the cervical nodes from above downward and in that of the thoracic nodes from below upward.

Grawitz[1] has also maintained the importance of a descending tuberculosis from the tonsils and cervical nodes to the tracheal and bronchial nodes and finally attacking the lungs. Aufrecht, Beckmann,[3] and others also lay the main stress on infection of the[2] tonsils.

[1] *Deutsche med. Wchnschr.*, 1901, 27. p. 711.

[2] *Verh. d. deutsch. path. Gesellsch.*, 1902, 4, p. 65.

[3] *Das Eindringen der Tuberculose und ihre Bekämpfung*, 1904.

Finally, one may have an ascending tuberculosis from the abdominal lymph nodes to the chest. The infection, which in such cases probably has taken place through the intestinal mucosa, first attacks the mesenteric nodes and extends to the retroperitoneal nodes and further to the various groups situated in the chest and neck. Up to the present time this route of infection has received little attention; in all likelihood, however, it is much more frequent than is commonly supposed. The frequency of latent tuberculosis of the mesenteric nodes, which has been demonstrated mainly by the English authors previously named and by Heller and his pupils, is suggestive in this connection, as are the recent findings of a Norwegian investigator, Hans Thue, who systematically examined the mesenteric nodes of children. It is notable that the more the investigations have been aimed at this mode of infection the more frequent have been the positive results. Numerous animal experiments also point this way, for instance, the highly interesting feeding experiments recently related by Weber and Bofinger.[1] On feeding tubercle bacilli from chickens to rabbits, mice, and guinea pigs the intestinal follicles, mesenteric and submaxillary nodes were first infected; after seven weeks the lungs became involved, and in the third month the bronchial nodes, etc. In the interpretation of these cases of disseminated lymphatic tuberculosis on the basis of extension from a single group we come to a cardinal point:

Can a pathological process, as we here have supposed, really pass from one group of lymph nodes to another? Or, on the contrary, does not each organ possess its definite lymphatic organs which are not connected with each other, but isolated?

This is a question of the greatest interest also in general pathology, in connection with the spread of various morbid processes, especially inflammatory conditions and tumors. It may be stated that although tuberculous inflammation, like many other morbid processes, is prone to remain localized within a certain part of the lymphatic system it has long ago been proved[2]

[1] *Tuberculöse Arbeiten aus d. kais. Gesundheit.*, 1904, 1, p. 83.

[2] C. Weigert, in particular, as early as 1884 (*Jahrb. f. Kinderheilk.* 1884, 21, p. 146), emphasized the communications between the lymphatic channels of different regions and their importance in the spread of tuberculous inflammation within the lymph vascular system.

that extension to other groups of lymph nodes and lymph vessels may take place comparatively easily in a direction opposite to that of the lymph current. This occurrence is facilitated when some lymph vessels have become obliterated; the extension will then take place through the capillary communications which exist between the various regions. In the formation of carcinomatous metastasis we also observe an upward extension by continuity through the lymphatics along the spine, subsequently reaching the nodes in the posterior mediastinum and extending further to the neck. Evidence in the same direction is also obtained by inoculating animals with tuberculous material, as has long been recognized, and recently again brought forward by Westerhoeffer (compare also the observations of Weber and Bofinger). The numerous subcutaneous inoculations of guinea pigs, which I have performed in connection with this work, have shown that the infection first has passed to the inguinal nodes, then to the retroperitoneal nodes and to the liver and spleen, thence upward to the posterior mediastinal and hilus nodes and finally to the supraclavicular and cervical nodes. Later the lungs become the seat of scattered tubercles, apparently by lymphogenous infection from the hilus nodes, or perhaps by way of the blood. This is the very route which we previously assumed that tuberculous infection might take in its upward extension from the abdominal cavity. I am quite convinced that infection in man not rarely takes place in this manner, and that as a result not seldom the picture of generalized lymphatic tuberculosis is obtained.

In the discussion of the locations invaded by tubercle bacilli we have started from the generally assumed belief that we may conclude from the localization of tuberculosis in certain definite lymphatic groups that the infection took place through the corresponding organs with their mucous membranes. This may also be accepted as the general rule and is supported by much pathological experience. It is, however, doubtful whether it always holds true. This question has become one of importance within the last few years since the claim has been made by v. Behring that most cases of tuberculosis in adults are attributable to infection in infancy and through the digestive tract irrespective

of the location of the later and most marked pathological changes. In discussing this matter we must first of all direct our attention to children infected during the first year, and especially during the first months of life. It appears to be the case—and of this I have become more strongly convinced the more I have occupied myself with this subject—that tuberculosis readily and rapidly invades the body of the child and quickly reaches those organs which we know from experience to be especially prone to tuberculous inflammation, namely the lymph nodes. One gets the decided impression that tuberculosis relatively easily finds its way to the various lymph nodes.

Most frequently the extension appears to be by the lymph stream but the tendency in children to rapid and frequent infection of the internal organs and to generalization of the tuberculosis also points to the blood stream as a frequent route; and as the lymph nodes, as often emphasized, must be considered especially susceptible, there is nothing against the assumption that they have been infected by way of the blood.

It is also of great importance to bear in mind that nothing appears to be in the way of assuming that tubercle bacilli may pass through one or more groups of lymph nodes before they become stationary and set up inflammation. Thus, nothing appears to me to prevent us from believing that tubercle bacilli may pass through the intestinal mucosa and not cause tuberculous changes until the retroperitoneal, or even the thoracic, lymph nodes have been reached. As stated, feeding of tuberculous material to animals has been known to produce tuberculosis of the nodes in the chest without simultaneous tuberculous lesions in the abdomen. Tubercle bacilli sometimes have been demonstrated in the thoracic duct shortly after the feeding.

If future investigation shall determine that the presence of latent tubercle bacilli is of frequent occurrence also in the mesenteric nodes, then the assumption would be considerably strengthened that the thoracic organs also may be infected by this route. It is shown by clinical observation that tuberculosis of the lungs and thoracic lymph nodes sometimes is preceded by enteritis of long duration. Positive assertions,

of course, cannot be made on this point, as one cannot clinically follow the course of successive swelling of the abdominal groups of lymph nodes.

This route of infection, however, ought to be borne in mind, and especially when the mesenteric and retroperitoneal lymph nodes and the thoracic viscera are diseased.

This question, on the whole, centers about the lymph nodes in the chest. Here another route which we have previously touched upon, comes into consideration, namely, a descending tuberculous infection from the cervical nodes. We have already argued in favor of the extension of a real tuberculous inflammation from the cervical to the thoracic nodes. But we cannot ignore the assumption that in certain instances tubercle bacilli which enter the cervical nodes (where they are quite frequently demonstrated as latent bacilli) may migrate to the nodes in the chest and there cause definite tuberculous lesions. It is sometimes observed that the disease begins with swelling of the cervical nodes which later decreases; the swelling extends downward along the cervical nodes, and later becomes associated with phenomena on the part of the thoracic lymph nodes and the lungs. In such a case one is hardly mistaken in assuming that a descending infection has taken place.

On the whole I believe that it must be admitted that in children as well as in adults a tuberculous infection as a rule is attended with local changes in the corresponding lymph nodes, but at the same time it must be emphasized that dissemination may take place rapidly and easily to the different systems of organs through the different natural vascular systems without the localization apparently being of particular importance.

THE FREQUENCY OF TUBERCULOSIS IN ADULTS.

As stated in the introduction, the work on tuberculosis in children gradually led me to consider tuberculosis in adults, its connection with the disease in childhood and its primary localizations, especially in the lymph nodes and intestines. Originally attention was mainly directed to the two latter points, but this gradually led to a search for tuberculous changes in general in the entire autopsy material. As, however, these investigations

strictly were beyond the scope of my research aud have been made somewhat desultorily by myself and my assistants we have not reached as high figures regarding the frequency of tuberculosis in adults as would be the case if we had been concerned exclusively with that side as were other investigators (Bugge, Nägeli, Lubarsch, Schmorl, Burkhardt, etc.). However, as my figures in their main features agree with those of others and serve to illustrate certain points I think they ought to be given.

For comparison I will first summarize the results from the four preceding years, 1897–1900, when no special effort had been made to demonstrate latent or obsolete tuberculous foci.

TABLE VI.

Year	Autopsies in Adults (over 15 years)	Without Tuberculosis	Total Number with Tuberculosis	Cases in which Tuberculosis caused Death	Cases with Latent and Obsolete Tuberculosis	Cases with Progressive Tuberculosis
1897....	169	68=40.2%	101=59.8%	43=25.5%	46=27.2%	12=7.1%
1898....	200	100=50.	100=50.	45=22.5	43=21.5	12=6.
1899....	195	102=52.3	93=47.7	35=17.9	48=24.6	10=5.2
1900....	172	91=52.7	81=47.2	44=25.6	27=15.7	10=5.9
Total..	736	361=49.05%	375=50.95%	167=22.7%	164=22.3%	44=5.95%

It is seen that here the number of cases of latent and obsolete tuberculosis is very small.

Table VII illustrates the distribution of the different tuberculous changes at different periods:

TABLE VII.

Age	Autopsies	Without Tuberculosis	With Tuberculosis	Cases in which Tuberculosis caus'd Death	Cases of Obsolete Tuberculosis	Virulent Tuberculosis
16-20.....	72	28=38.9%	44=61.1%	30=41.6	4	10
21-25.....	87	30=34.3	57=65.5	39=44.9	16	2
26-30.....	80	36=45.0	44=55.0	31=38.8	12	1
31-35.....	71	30=42.3	41=57.7	21=29.6	14	6
36-40.....	70	37=52.9	33=47.1	12=17.1	14	7
41-45.....	52	30=57.7	22=42.3	8=15.4	13	1
46-50.....	74	39=52.7	35=47.3	10=13.5	18	7
51-55.....	54	39=72.2	15=27.8	4= 7.4	9	2
56-60.....	52	28=53.8	24=46.2	7=13.5	14	3
61-65.....	44	22=50.0	22=50.0	0	21	1
66-70.....	26	12=46.2	14=53.8	4	9	1
71-75.....	21	12=57.2	9=42.8	1	8	0
76-80.....	19	8	11	1	10	0
81-85.....	4	1	3	0	2	1

In certain respects the recorded findings are different in the years 1901–3, during which the investigations of tuberculous children were made. Altogether 537 adults (over 15 years) came to autopsy, divided as follows:

TABLE VIII.

	Autopsies in Adults	Without Tuberculosis	With Tuberculosis	Fatal Tuberculosis	Latent and Obsolete Tuberculosis	Progressive Tuberculosis
1901	169	49=29 %	120=71 %	36=21.3%	78=46.2%	6=3.5%
1902	203	64=31.5	139=68.5	52=25.5	73=36	14=7
1903	186	59=31.7	127=68.3	36=19.4	81=43.5	10=5.4
Total..	558	172=30.8%	386=69.2%	124=22.2%	232=41.6	30=5 4%

Table IX gives the distribution at different ages as to number of cases and in percentages based on the total number of deaths in each class:

TABLE IX.

Age	Autop-sies	Without Tuberculosis	With Tuberculosis	Died of Tuberculosis	Obsolete Tuberculosis	Virulent Tuberculosis
16–20.... ...	65	18=27.7%	47=72.5%	29=44.6%	16=24.6%	2= 3.1%
21–25.....	65	19=29.2	46=70.8	30=46.2	15=23.1	1= 1.5
26–30.....	57	20=35.1	37=64.9	18=31.6	17=29.8	2= 3.5
31–35.....	43	15=34.9	28=65.1	9=20.9	17=39.5	2= 4.7
36–40.....	49	15=30.6	34=69.4	11=22.4	19=38.8	4= 8.2
41–45.....	52	20=38.5	32=61.5	8=15.4	22=42.3	2= 3.8
46–50.....	49	18=36.7	31=63.3	2= 4.1	25=51.0	4= 8.2
51–55.....	44	17=38.6	27=61.4	5=11.4	20=45.5	2= 4.5
56–60.....	37	9=24.3	28=75.7	3= 8.1	22=59.5	3= 8.1
61–65.....	33	11=33.3	22=67.7	6=18.2	15=45.5	1= 3.0
66–70.....	30	3=10.0	27=90.0	0	23=76.7	4=13 3
71–75.....	22	5=22.7	17=77.3	2	12	3
76–80.....	7	2=28.6	5=71.4	0	5	0
81–85.....	1	0	1	0	1	0
86–90.....	3	0	3	0	3	0

The following chart shows these results as far as these figures can be represented in curves, *i*, *e*., until the figures become too small to be graphically represented.

As is seen, the curve of deaths from tuberculosis in different five year periods is highest at the ages of 16–20 and 21–25 years, the figures here reaching 46 per cent of all deaths. Later the curve rapidly and evenly declines, the minimum death-rate of four

per cent being reached in the period 46–50 years, slightly rising again at the age of 60 to 70.

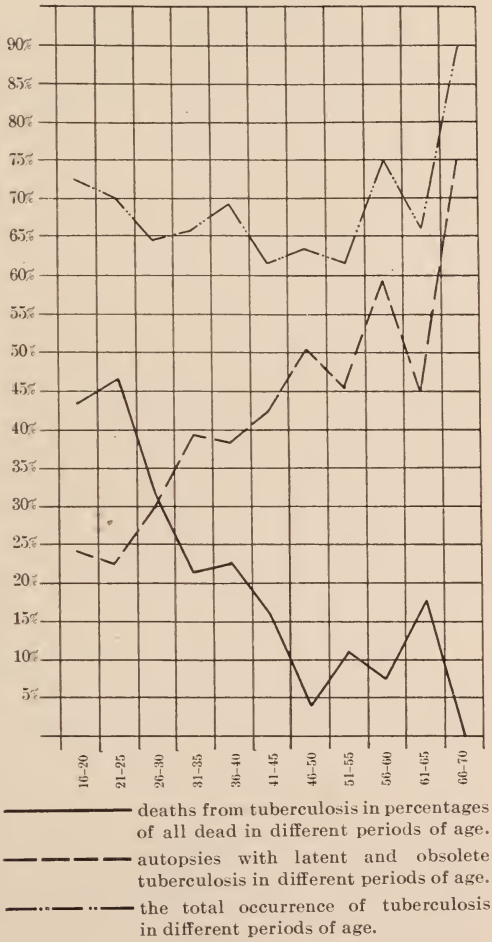
The curves of the latent and obsolete cases show quite opposite conditions, being lowest at the ages between 16 and 25 years (23 per cent). Later it rises rapidly and at 31–35 years reaches 40 per cent, at 55–60 about 60 per cent, and at 66–70 about 77 per cent.

When all tuberculous changes put together are represented by one curve it begins comparatively high at the age 16–20, with 72.5 per cent, then sinks somewhat to the forties (61.5 per cent), again rising rapidly in the sixties, where it reaches 90 per cent.

In connection with these curves it must be remarked that the absolute numbers from which the percentages have been computed are quite small between the ages of 60 and 70. On the whole, however, these curves must be considered correct expressions of the relationship of fatal tuberculosis on the one hand and of the latent and obsolete forms on the other hand at various ages.

CHART I.

FREQUENCY OF TUBERCULOSIS IN ADULTS.



As we see, the figures for the years 1901–1903 are considerably higher than those of the preceding period 1897–1900, and

the increase is due to the much larger number of latent and obsolete[1] cases included, while the number of those who died of tuberculosis is about the same and in accordance with the mortality of tuberculosis in the entire country. Yet, several cases may have escaped attention while all those included may be considered as certain. The cases with only pleural adhesions, small cicatrices and indurations in the lungs without pultaceous or caseous foci, plaques of thickened pleura over the apices, etc.—changes often noted in the autopsies, have not been included. Moreover it must be remembered that microscopic examination of doubtful foci was only exceptionally made. If we also consider that negative findings have been recorded in cases with non-tuberculous lesions in the lungs, such as pneumonias, tumor metastases etc., it may be concluded that the figures quite certainly ought to have been greater. Arbitrarily, I believe, the percentage of tuberculous changes might have been brought up to 75 or 80 or more.

By way of comparison, it is of interest to recall the figures obtained by another Norwegian observer, J. Bugge,[2] which are based on extremely exact observations. Bugge obtained about the same figures; his classification of the various tuberculous changes, however, is somewhat different.

In 200 autopsies of individuals over 14 days old (21 under one year and 22 from one to nine years) Bugge, who exclusively directed his attention to the lungs and bronchial nodes, found tuberculous changes in 136 cases, or 68 per cent while 64 or 32 per cent were free from tuberculosis of the lungs and bronchial nodes. Of the 136 cases, in 42 (21 per cent) death was due to tuberculosis, in 45 (22.5 per cent) there was virulent tuberculosis (demonstrated by inoculation of guinea pigs), and in 49 (24.5 per cent) obsolete, healed tuberculosis.

The difference between Bugge's statistics and mine will be noted. In my tables for the years 1901–3, cases of developing tuberculosis, *i. e.*, virulent and progressive tuberculosis, have been grouped together, and in another group have been placed the cases of latent and obsolete (always encapsulated) tuberculosis, which constitute the bulk of the whole number. This was necessary as I did not make inoculations, and hence could not decide what latent foci did or did not contain virulent bacilli. The question whether in Christiania conditions are such as to be correctly represented by Nägeli's figures, according to which practically all adults show traces of tuberculosis, I shall leave unanswered. However, it appears unlikely to me, as we occasionally

[1] As latent we have considered those cases in which the process had not been completed and where we must suppose that virulent bacilli still exist. As obsolete we have considered cases of healed tuberculosis with contracted scar tissue, calcification, etc.

[2] *Undersögelser om Lungetuberkulosens Hyppighed og Helbredelighed*, 1896. Bugge made microscopic examination and inoculation of guinea pigs in all doubtful cases.

meet with cases in which the most searching examination fails to reveal tuberculous changes.

For comparison the statements concerning the frequency of tuberculosis in certain more extensive recent works will be given.

Nägeli[1] in his first series found 75 per cent, later 90 per cent of tuberculosis in adults (over 17 years), but in his most recent series the percentages rose to 97 and 98. In his last 284 autopsies there were only six in which no trace of tuberculosis was found; among these 284 cases there were 63 lethal forms, 74 active latent. 111 inactive latent (healed); 32 were in a very uncertain position.

Nägeli reaches his high figures by including in the inactive latent forms apical pleural adhesions, pleuritic scars with shingle-like indurations, calcified foci in the lungs and bronchial nodes, and combinations of these findings.

Although most of the lesions included probably were tuberculous yet there is doubt in regard to some of the cases, for instance, the shingle-like indurations. This is also partly admitted by himself. The correctness of this objection is also shown by the investigations made by Lubarsch. (See article cited below).

Burkhardt,[2] in his report of 1262 autopsies in adults over 18 years from the pathological institute of Dresden (Prof. Schmorl) also reached very high figures, tuberculosis in some form being present in 91 per cent of the cases. There were 466 cases (41 per cent) of death from tuberculosis, 209 cases of latent active forms, and 474 cases of latent inactive forms. It is seen that it is the very high percentage of lethal cases (41 per cent) which causes the high total of 91 per cent, while the figures in the other two classes about correspond with those of myself and others. On account of this anomaly, which is supposedly due to the material Burkhardt's figures cannot be compared directly with the others.

According to Hof's[3] account of 15,000 autopsies from Kiel, tuberculosis was found in only 2,697 of the 7,683 autopsies in adults (35.1 per cent).

Lubarsch, in his interesting work previously mentioned, obtained considerably higher figures. There were about 1,820 autopsies (297 in children), and tuberculous changes were found in 1087 cases, and, if some (about 10 per cent, of the shingle-like indurations are included, in 1,106 cases (60.6 per cent) On deducting persons under 10 years we have tuberculosis in 1,040 out of 1,522 cases, or in 69.1 per cent, which is quite close to my result. Among the 1,087 cases there were 515 (47.4 per cent) of florid tuberculosis, 485 (44.6 per cent) absolutely healed, and 86 (7.9 per cent) almost healed — the last two groups together constituting 52.5 per cent. These figures also correspond quite well to mine.

It is still too early to express one's self as to what might be considered the usual percentage of tuberculosis in the dead. The figures of Lubarsch and myself perhaps are too small; however, I believe on the other hand that those of Nägeli and Burkhardt are

[1] *Virch. Arch.*, 1900. 160, p. 426–472.

[2] *Münch. med. Wchnschr.*, 1903, 29, p. 1275.

[3] *Loc. cit.*

too high. Some autopsies always will occur in which no trace of tuberculosis is found no matter how careful a search is made, even where microscopic examinations and inoculations as made by Bugge, are employed. Otherwise it may well be that conditions vary greatly in different places.

THE PRIMARY LOCALIZATION OF TUBERCULOSIS OUTSIDE THE RESPIRATORY TRACT IN ADULTS, WITH SPECIAL REFERENCE TO ITS OCCURRENCE IN LYMPH NODES AND IN THE INTESTINAL TRACT. TUBERCULOSIS OF LYMPH NODES IN ITS RELATION TO PULMONARY TUBERCULOSIS IN ADULTS.

I wish first to consider my experience with tuberculosis in adults in the years 1897–00, *i. e.*, before the inauguration of systematic study of the entire material. It seemed little likely that much of interest would be found in this material, yet many cases elucidate lymphatic tuberculosis in general as well as its mode of extension and duration. Hence some examples of the more characteristic forms are worth including.

On perusing the records of the 375 cases from the years 1897–00 in which tuberculosis was found (51 per cent), and in 167 (22.7 per cent) of which death was from tuberculosis, I find only nine cases in which there is a history of enlarged lymph nodes in childhood, remnants of which, at times, were found at autopsy in the form of scars on the neck. This figure naturally is much too small, as this condition is not always looked into and information concerning it not always recorded in the clinical history. In six of the nine cases no connection between these enlarged nodes in childhood and the subsequent pulmonary tuberculosis could be demonstrated anatomically, as there was no trace of a progressive lymphatic tuberculosis, the pulmonary tuberculosis apparently having developed as a primary disease. In the three other cases, on the contrary, there was quite an extensive, in all probability primary, lymph node tuberculosis, in two cases chiefly located in the chest and giving rise in the one case to miliary tuberculosis, in the other to pulmonary tuberculosis.

In regard to the third case, that of a woman 24 years old, it was stated that when quite little she had "glands in the eyes," at ten "glands in the stomach," and at thirteen "glands in the neck,"

and that she was operated on for the latter affection at 18. She died at 24 after an illness of half a year. Aside from comparatively recent pulmonary tuberculosis and small intestinal ulcers, there was quite marked tuberculosis of the lymph nodes which were enlarged to walnut-size, caseous, and partly calcified; the disease was most marked in the thorax (tracheal and hilus nodes) and in the mesentery.

The lymph node affection may here quite certainly be considered primary, and the clinical history would suggest a primary descending tuberculosis of the cervical nodes.

Otherwise, in the 17 cases (plus the three cases just mentioned) there was a primary and a well advanced tuberculosis of the nodes in the chest (especially in the tracheal and hilus nodes) with secondary tuberculosis in other organs, viz., miliary tuberculosis in nine cases, sometimes most prominent in the lungs, tuberculosis of the serous membranes in two cases, and in the other six cases secondary pulmonary tuberculosis. (I leave out the numerous instances of latent and obsolete lesions of the thoracic nodes.)

To these must be added two cases of extensive, almost general, lymph node tuberculosis (in one case mainly abdominal, perhaps primarily so; it will be considered later) with secondary pulmonary involvement. The affection of the lymph nodes was here very marked and old, most of the nodes being caseous, while the pulmonary lesions were relatively recent.

Thus we have 12 cases of pulmonary tuberculosis in which we have every reason to look upon it as secondary, it having appeared subsequently to, and probably from, tuberculous nodes.[1] In a few cases perforation from bronchial nodes to the bronchi was demonstrated, in others the pulmonary tuberculosis must be looked upon as of lymphogenous or hematogenous origin, of the latter especially when the original seat was extrathoracic.

If six cases of primary involvement of the mesenteric and retroperitoneal nodes and finally one case of primary tuberculosis of the axillary and cubital nodes are added it will be seen that primary lymph node tuberculosis is quite frequent in adults

[1] In addition there were cases of small encapsulated foci in the lungs in connection with primary tuberculosis of other organs, to be referred to later.

—altogether 28 cases—and that its rôle is quite important as it most frequently causes death from secondary tuberculosis of vital organs. Most of these cases, therefore, belong to the 167 fatal cases, comprising about 15 per cent.

Certain cases merit brief narration:

Autopsy 176, 1899. Woman, aged 45, who one and one-half years before death had repeated attacks of rheumatic pain in the left arm. A year later she noticed swelling of the left cubital and axillary nodes. She died suddenly of pulmonary embolism from thrombosis of the veins in the arm. The autopsy revealed numerous wholly caseous lymph nodes, some of which were of walnut size; they were located beneath the outer end of the left clavicle, whence they formed a continuous chain to the axilla, and also along the entire arm, and at the elbow. There was no wound or scar on the left hand or arm. The tonsils, the cervical, thoracic, and mesenteric nodes were not tuberculous, but a recent peritoneal tuberculosis was present.

The affection of the left axillary and cubital nodes must here be considered primary; how this infection had been brought about remains unknown.

In regard to primary tuberculosis outside the respiratory organs and lymph nodes the conditions for the years 1897–1900 were as follows:

Primary tuberculosis of the genital organs in four cases (in some of these there were small encapsulated pultaceous foci or scars in the lungs or lymph nodes); of the urinary organs in one case; of serous membranes in two cases; of bones and joints in one case; of the skin in one case. In three cases there was tuberculosis of the adrenals, but always secondary, originating in the lungs or bronchial nodes.

The case of primary cutaneous tuberculosis is worth narrating on account of the subsequent descending affection of the lymph nodes.

Autopsy 104, 1898. Man 49½ years old. His wife and one daughter died of tuberculosis; eight years ago he developed lupus of the left ear, later swollen cervical nodes for which he was operated upon several times. Subsequently he developed tuberculosis of the pharynx and ear. He died with cerebral symptoms. Autopsy: In addition to lupus of the left ear there were tuberculous cervical nodes which were enlarged to walnut size and caseous, in part much softened; on the left side these softened nodes are confluent and formed an abcess the size of a pigeon's egg, which extended to the base of the skull. Along the entire trachea and in the posterior and anterior mediastinum there were numerous caseous nodes varying in size from that of a pea to that of a

hazelnut. In the apices of the lungs were several firm consolidated nodules up to walnut size, with caseous and pultaceous foci; also numerous tubercles of the size of a pin head or hemp seed, scattered or arranged in groups. Also tuberculosis of the left middle ear and tuberculous meningitis; one large and several small tuberculous ulcers in the ileum. In the root of the mesentery were several partly softened lymph nodes enlarged to the size of a walnut. Scattered tubercles in the liver, spleen, and kidneys.

It may be supposed that a descending lymphatic tuberculosis originated from the lupus on the left ear; and as the tuberculosis of the lymph nodes appear to be oldest and most pronounced the pulmonary involvement was probably secondary.

The cases of certain or probable primary infection through the intestinal tract must be given special mention. There were five cases. three of which were certain, two somewhat doubtful.

1. In one case (128, '98) there were scars in the small intestine which had to be looked upon as signs of healed and isolated intestinal tuberculosis.

2. In a second case (56, '98) there was a comparatively recent intestinal tuberculosis and also a scar in the apex of one of the lungs. In all probability the intestine had been separately infected and probably considerably later than the lung in which the process was inactive and completely encapsulated.

3. A third case (154, '00) concerned a woman 40 years of age who ten years before death had been operated on for tuberculosis of the wrist. She died of miliary tuberculosis. The autopsy revealed the scar of a tuberculosis of the left wrist (healed after operation); tuberculosis of the lymph nodes in the chest; a few encapsulated caseous foci in the apices of the lungs of the size of grains of wheat; miliary tubercles, especially in the lungs and kidneys; large tubercles of the cerebellum, spleen, and liver; finally large, transverse, old ulcers of the large intestine and caseous mesenteric nodes. The intestinal tuberculosis cannot readily be explained except by assuming a primary infection of the intestine.

4. The following case must be looked upon as one of primary tonsillar and intestinal tuberculosis (39, '99.) A girl, 17 years old, entered the hospital in an unconscious condition and died shortly after admittance. There was miliary tuberculosis of all organs, including the lungs, in which, otherwise, only an encapsulated, caseous nodule, the size of a pea, was found in the right lower lobe. There was a caseous nodule in the thoracic duct. Right tonsil rather large and in an area .5 c.m. wide, coated with yellowish material; it contained masses of tubercles. On the right side of the neck were lymph nodes of the size of beans; one node, of hazelnut size, was caseated: microscopically, masses of tubercles. In the hilus of the lungs enlarged nodes, in part studded with tubercles; there was no continuous chain of nodes along the trachea and neck. In the intestine scattered tuberculous ulcers; in the mesentery enlarged caseous nodes. Beginning tuberculous salpingitis.

The oldest tuberculous changes appear to be in the digestive tract, viz., in the tonsils and, more marked, in the intestines; the

involvement of the latter, at any rate, is not secondary to tuberculosis of the respiratory tract.

5. The following is a pure case of tuberculosis of the intestines and mesenteric nodes (232, '98). A woman, 45 years old, died of fecal fistula. There were large tuberculous ulcers in the cecum and ascending colon, and numerous swollen tuberculous lymph nodes in the mesentery and retroperitoneal connective tissue, especially around the pancreas. No trace of involvement of the lungs. In the hilus nodes, on the other hand, tubercles were demonstrable microscopically.

There were also nine cases in which primary tuberculosis of the mesenteric and retroperitoneal nodes was demonstrated, in three cases associated with involvement of other nodes, but from its appearance older and more marked than the latter affection; in six cases the abdominal nodes only were involved. The lymph nodes attacked were always caseous. In these cases we may assume a primary infection through the intestinal tract, at any rate when the mesenteric nodes were involved; the matter is somewhat more in doubt when the retroperitoneal nodes alone were involved (as was true of three cases, in one of which there was a secondary adrenal tuberculosis).

Of the cases just mentioned only one will be narrated which quite certainly must be looked upon as an ascending affection of lymph nodes originating in the abdomen.

(67, '99.) Woman, 19 years old. At 13 abdominal pain and diarrhea set in, followed by abdominal enlargement and emaciation; the following summer she entered a hospital on account of "glands in the stomach;" she could feel hard lumps herself. After 15 months she left and was quite well. Three months before death abdominal pain recurred and was accompanied by fever. The autopsy revealed both old and recent tuberculous peritonitis. No intestinal ulcers. Very extensive lymph node tuberculosis in the mesentery, retroperitoneal connective-tissue, upward along the spine (a cluster the size of a fist behind the stomach), at the roots of the lungs; bean-sized supraclavicular nodes on both sides; tonsils atrophic. Lungs entirely free from tuberculosis.

We have here a primary lymph node tuberculosis which we must assume to have originated in the mesenteric nodes and to have ascended.

Hence, there were altogether 14 cases (nine certain, five somewhat doubtful) of primary infection of the digestive tract (intestine, abdominal nodes, tonsils). This constitutes only about two

per cent of the total 736 autopsies, and about four per cent of the 375 autopsies in which tuberculous changes had been demonstrated. If to these cases we add those of primary localization outside the respiratory and digestive tracts and some of the primary lymphatic cases, the bulk of the remaining cases depends on primary infection through the respiratory tract, which in the great majority of the cases—at least 90 per cent—is primarily attacked. However, in most of the latter cases, the lesions consist of tuberculous inflammation of lungs and thoracic nodes which is often completely healed. On the other hand, the cases in which the disease originated outside the respiratory tract most frequently terminated fatally and really should be considered together with the 167 cases of fatal tuberculosis. Thus the importance of these cases is enhanced. But, on the whole, the number of recorded cases of primary tuberculosis outside the lungs and lymph nodes in this series is too small; among other reasons because sufficient attention had not been given to detecting such cases.

For the years 1901–03 conditions are decidedly different. During this period the bodies of 538 adults came to autopsy, 124 (or 22.2 per cent) had died of tuberculosis while 232 (or 41.6 per cent) had latent or obsolete tuberculosis.

The number of cases of supposedly primary lymph node tuberculosis here is quite great.

Of primary tuberculosis of the hilus, tracheal and bronchial nodes[1] seven cases were observed, most of which occurred in young persons (ages respectively 16, 17, 17, 17½, 20, 31, 74 years) illstrating the well-known fact that from the age of 15 and a little into the twenties tuberculosis as a rule is a primary lymph node affection. Secondarily this had given rise to miliary tuberculosis (two), tuberculosis of the genital organs (one), of the lungs (one), of bone (one), of serous membranes (two).

There were 10 cases at least—four of them somewhat doubtful—of primary tuberculosis of the cervical nodes with subsequent extension to other lymph nodes and to internal organs the

[1] The numerous cases of small, encapsuled latent or obsolete tuberculous lesions in these nodes are not included.

involvement of some one of the latter being the cause of death in most cases.

Further, there were some cases of generalized tuberculosis of lymph nodes in which the point of origin no longer could be demonstrated; some of the cases appeared to be instances of descending tuberculosis from the throat. In some cases there was tuberculosis in two different groups, the connection of the lesions not being cleared up (in cervical and thoracic nodes or in thoracic and abdominal nodes).

If we also add the cases of isolated or primary tuberculosis of the mesenteric or retroperitoneal nodes the total number of cases of well-marked, primary tuberculosis of lymph nodes will be 30. In most of these cases death was due to subsequent localization in internal organs, so they form a considerable proportion, about 20 per cent of the total deaths from tuberculosis. Herein we must include a small group of six cases in which there was considerable lymph node tuberculosis but also advanced involvement of various internal organs, mainly the lungs so the connection becomes somewhat doubtful.

To illustrate this class I shall narrate several cases, especially of the generally neglected type of progressive tuberculosis of the cervical nodes, and of general tuberculosis of the entire lymph node system. The clinical features will also be briefly stated whenever necessary for the understanding of the evolution of the tuberculous changes.

Case 1. (124, '01.) Girl, 19 years old, died of amyloid disease on June 26, 1901. There was a pronounced family history of tuberculosis. She had bronchitis and twice pneumonia in childhood. From the age of five years there was enlargement of the cervical nodes. At seven diarrhea with painful bloody stools. She was in the hospital from January 1895 to January 1896, and a diagnosis of tuberculosis had been made. There were lumps of the size of a hen's egg in the neck, above the clavicles and in the axillae and groins; also phlyctenular kerato-conjunctivitis. Enlarged liver and albuminuria (amyloid degeneration). Autopsy: Clusters of nodes of goose-egg size on both sides of the neck. Lungs normal. The nodes along the bronchi, at the hilus of the lungs, and along the trachea were somewhat enlarged: in most of them calcareous or pultaceous material was not seen. The mesenteric nodes and — to a lesser degree — the retroperitoneal nodes were enlarged and generally contained pultaceous foci. No intestinal ulcers, but tuberculous salpingitis and endometritis were present. Amyloid degeneration of all organs.

Undoubtedly the lymph node affection here was primary and originated in either the cervical or mesenteric nodes (or possibly, in both groups). The clinical history points to the cervical nodes as the primary seat.

Case 2. (26, '02.) Servant girl, 40 years old; enlarged cervical nodes since the age of one and one-half years. At 23 she was operated on for enlarged glands on both sides of the neck, under the chin, and in both axillae. Later she was apparently well until digestive disturbance set in about a year before death. In March 1901 pain in the ileocecal region and bloody stools suddenly appeared. The autopsy revealed an almost general tuberculosis of the lymph nodes, viz., in the nodes in the lower part of the neck (not the tonsils); along the trachea, increasing downward: in the nodes in the hilus of the lungs (but not in the bronchial nodes), in the retroperitoneal nodes (in hilus of liver and spleen); in the mesenteric nodes, decreasing in size and number toward the intestine (largest and most numerous in the root of the mesentery). The retroperitoneal nodes formed a large mass of pultaceous and caseous nodes, up to the size of a pigeon's egg, which adhered to the duodenum. A large node had caused a narrowing of the lumen of the duodenum (with secondary dilatation of the stomach), and finally had perforated the wall of the duodenum and produced a tuberculous ulcer. No tuberculosis of the nodes in the small pelvis or in the inguinal nodes. No intestinal ulcers (except the one in the duodenum). In the apices of the lungs small encapsulated caseous nodules.

The clinical and postmortem findings here point to a descending affection from the cervical nodes to the tracheal and probably further to the retroperitoneal nodes. The small encapsulated foci in the lungs must be looked upon as secondary, either brought about by hematogenous infection from the lymph nodes, or as having arisen at another time (possibly by inhalation of tubercle bacilli).

Case 3. (72, '02.) Man, aged 30, who at 15 developed enlarged cervical nodes which steadily grew larger and were operated upon. In 1898 one node began to suppurate, and later had discharged pus from time to time. During the winter of 1897–8 he had a prolonged bronchitis, and pleuritis in the summer '99. In December '01 again cough, later hemoptysis; he died April 17, '02. Autopsy: Pulmonary and intestinal tuberculosis of usual appearance (cavities in one apex). Extensive lymph node tuberculosis with greatly enlarged caseous nodes along the neck from the mastoid processes to the clavicles; along the trachea, especially at its bifurcation, in the hilus of the lungs, the retroperitoneal tissues, in the mesentery and groins. The nodes were enlarged to the size of pigeon's eggs, partly softened.

On the basis of the clinical data we may suppose that the affection of the lymph nodes was primary (probably originating in the

cervical nodes), and that the pulmonary tuberculosis appeared later, possibly on the basis of the lymphatic affection.

Case 4. (170, '02.) A woman, aged 21; as a child she had enlarged cervical nodes (which did not suppurate), and chlorosis at 10; otherwise well until two attacks of appendicitis at 19; since then she was not quite well, was especially troubled by constipation, felt weak, and steadily lost in flesh. After the appearance of edema and increasing marasmus she died September 15, 1902. Autopsy: Extreme emaciation; pronounced enteroptosis; also anemia and considerable atrophy of all organs. Bilateral bronchopneumonia. Tonsils small, without scars. Cervical lymph nodes on both sides much enlarged, forming chains of completely caseated nodes from hazelnut to walnut size (a softened few nodes at the back of the neck). They continue downward in both supraclavicular regions; in the posterior mediastinum along the esophagus and aorta where there are shrivelled calcareous or pultaceous nodes of hazelnut size; smaller nodes in the pulmonary hilus (not along the bronchi). In the apex of the right lung three small encapsulated caseous or calcareous nodules. In lower ileum, in the cecum and part of the ascending colon there are old contracted tuberculous ulcers with marked thickening of the intestinal walls and adhesions to surrounding structures. In the mesentery a number of pea-size calcareous nodes; likewise in the retroperitoneal connective tissue above the pancreas, and in the hilus of the liver and the lesser omentum. A direct connection with the posterior mediastinal nodes could not be demonstrated.

The clinical and postmortem findings here are decidedly in favor of assuming an old primary tuberculosis of the cervical nodes, descending to the supraclavicular and posterior mediastinal regions (and possibly to the retroperitoneal nodes). Then the intestinal tuberculosis must be looked upon as an old primary tuberculous affection. The small nodules in the lungs are most probably secondary, due to hematogenous infection in an organism, a whole system of which, namely, that of the lymph nodes, for years had been permeated by tuberculosis. But an independent infection of the lungs by inhalation cannot be excluded.

Case 5. (64, '03.) Man, 23 years old. Mother died of pulmonary tuberculosis, and another of her children had enlarged nodes. At the age of 10 or 11 years he developed enlarged cervical nodes which suppurated from time to time, and persisted; two years ago the nodes on the right side of the neck were operated on. Otherwise healthy. Four months before death cough, pain in the side, and signs of tuberculosis of the thoracic organs. He died April 5, 1903. Autopsy revealed a very extensive tuberculosis, with calcification and caseation, and partial fibrous transformation of the lymph nodes, viz., cervical of both sides, axillary, posterior and anterior mediastinal, hilus and bronchial, retroperitoneal and mesenteric. Also tuberculosis of lungs and pleurae, and finally miliary tubercles in spleen, liver, and kidneys.

It may be assumed with certainty that the lymph node affection was primary, descending from the neck to chest and abdomen. Probably the origin of the pulmonary tuberculosis also lay here; but it may also have arisen independently and later, in another manner (inhalation?).

Case 6. (111, '04.) Woman, aged 48, who died of amyloid degeneration and extensive thromboses. As a child she had scarlet fever, measles, and "glands of the neck" (not suppurating). At 36 repeated attacks of cholecystitis. Four years before death bronchitis set in. The autopsy revealed an old tuberculosis of the cervical nodes. Below angle of right jaw an almond-sized completely fibrous node with a pultaceous focus in its middle. Outward, along the right side of the neck to the clavicle there was a continuous fibrous lymphatic chain with a row of hard, partly pultaceous nodes up to bean size. Similar nodes along the trachea, in the hilus pulmonis (no large ones at bifurcation), along the bronchi. Old pulmonary tuberculosis with cavities having smooth lining and surrounded by indurated fibrous tissue. No intestinal ulcers. Mesenteric nodes small, greyish red, soft

The clinical history in connection with the postmortem findings point to descending tuberculosis of the cervical nodes, and it seems natural to consider the pulmonary affection as dependent on it; but it might also have arisen independently later.

Case 7. (210, '91.) Woman aged 19, who died after an illness of about six weeks. The disease behaved like a serious infection (as typhoid fever or pyemia), with continuous high fever (temperature 100.4° to 104.5° F.) and increasing marked anemia. The autopsy revealed extensive and advanced tuberculosis of the lymph nodes, especially along the whole spine, viz., in those in the posterior mediastinum, along the trachea, in hilus pulmonalis, along the bronchi, in the retroperitoneal, iliac, inguinal (size of pigeon's egg), and mesenteric nodes, the latter decreasing in size toward the intestine. The nodes were completely caseous, in part softened; the posterior mediastinal and retroperitoneal partly calcified. The axillary and cervical nodes were comparatively little swollen. There was rupture of a bronchial node into a bronchus leading to the right middle lobe, in which scattered tuberculous bronchopneumonic foci were seen. Tubercles here and there in the spleen and liver. No tuberculous ulcers in the intestines.

The disease of lymph nodes must be considered primary, that of the lung secondary. It cannot be settled whether the lymph node affection originated in the thoracic or abdominal nodes. The clinical picture with anemia and prolonged fever is of great interest.

Case 8. (127, '02.) Man, 56 years old, who died with a peculiar clinical picture; no information about "glands." The autopsy revealed large masses

of tuberculous nodes in the abdomen (retroperitoneal and mesenteric), along the entire trachea and in the posterior mediastinum, and also on both sides of the neck; the nodes were walnut- to pigeon's egg-sized and caseous. In one apex a cicatricial nodule with small calcareous and pultaceous deposits; in the liver numerous large tuberculous nodules.

The lymph node affection is very old; whether primary in the neck, chest, or abdomen cannot be decided.

Case 9. (129, '02.) Man, aged 43, of a tuberculous family. Symptoms of pulmonary tuberculosis for one year; a mass had been felt in the abdomen. Autopsy: Pulmonary tuberculosis with cavities. Also a continuous mass of large caseous lymph nodes in the mesentery, retroperitoneal tissues, posterior mediastinum, along the trachea and in the neck. Apex of left lung (and partly that of the right) surrounded by and embedded in a mass of caseous nodes, which were partly soft, partly hard, and enlarged to the size of a pigeon's egg. A few small recent tuberculous ulcers in the intestine. Scars on the neck. Tonsils normal. Very large and caseous inguinal nodes.

The affection of the nodes is at least very old; comparing it with the lung findings, it seems likely that the disease began in the lymph nodes. The scars on the neck point to the existence of an old tuberculosis of the cervical nodes.

Case 10. (152, '02.) Woman, aged 63, who had suffered for about one and one-half years from an itching skin disease, with infiltrations of the skin and desquamation (diagnosed by Professor C. Boeck as dermatitis exfoliativa universalis);[1] gradually marked enlargement of all lymph nodes appeared, bronchitis (without tubercle bacilli in the sputum), slight febrile attacks; finally diarrhea (no abnormalities in the blood). Died August 23, 1902. Autopsy: skin everywhere spotted, grayish brown, mostly smooth, in places desquamating; the spots vary in size from that of a hemp seed to that of a pea. Dr. Bruusgaard succeeded in demonstrating tubercles and tubercle bacilli in the spots. There were bronchitis and bronchopneumonia, but no sign of tuberculosis in the lungs. The lymph nodes at the root of the lungs were of hazelnut size, as were the mediastinal, axillary, and cervical nodes of both sides; some of the nodes were three to four c.m. long, 1.5 c.m. broad, soft, some of them almost broken down, with greyish white cut surface, on which numerous small yellow necrotic foci were seen. The mesenteric nodes were comparatively little enlarged, the retroperitoneal and inguinal nodes, on the other hand, enlarged (to the size of a pigeon's egg), and with small necrotic areas. A small ulcer in the ileum. On microscopic examination tubercles were found in the liver and spleen, and in the lymph nodes an atypical tuberculous granulation tissue rich in tubercle bacilli.

We have here an almost general tuberculosis of the lymph nodes and of the skin. Which is primary is not easy to state,

[1] The case has been described by Dr. Bruusgaard in *Norsk Mag. f. Laegevid*, 1903, 5, R. i., p. 156.

though probably that of the lymph nodes. Whether the latter affection was general from the outset or commenced in a certain region cannot be determined.

On perusing the cases just given,[1] one is compelled to admit that the primary localization of tuberculosis in adults frequently is in the lymph nodes, and that this form often has an extremely chronic course, with pronounced tendency to extension from place to place, from one group to another, and ultimately in the course of many years is prone to become almost general. Naturally, doubt will exist as to the conception of many of these extensive or general affections of lymph nodes. My opinion, as based on the combined postmortem and clinical findings, has been given in the epicritical remarks accompanying each case, and I find no reason to enter into further discussion of each case.

It is, on the other hand, necessary, on the basis of the entire material, to make a few remarks as to the localization and mode of extension of the infection in these cases in general. The explanation here can be in one of three directiens (compare what has been said in connection with tuberculosis in children):

1. Large portions of the lymph node system may be infected simultaneously with tubercle bacilli brought by way of the blood channels and deposited in the lymph nodes as localities predisposed to infection. The cases of fairly evenly distributed tuberculosis of lymph nodes without coexisting changes in the internal organs, especially in the mucous membranes, are the ones which might invite such interpretation. (Some are found among the 10 cases, for example, Nos. 8, 10, etc.) The infection might then be imagined to have taken place before birth through the placental circulation, and this possibility cannot be excluded, especially in cases of young persons with tuberculous affections dating back to the first years of life, and perhaps particularly in cases in which the chief localization is in the lymph nodes of the hilus of the liver, the retroperitoneal nodes, etc. Or, the

[1] It is also well to bear in mind certain cases from the years 1897-1900, such as 39, '99 (tuberculosis of tonsils and cervical nodes); 110, '00 (old tuberculosis of cervical nodes); 104, '98 (lupus with descending lymph node tuberculosis); 67, '99 (ascending tuberculosis from the abdomen). In the following some of the cases of intestinal tuberculosis, Nos. 6, 7, and 8, will also be taken into consideration.

infection may have taken place post partum, a considerable number of tubercle bacilli having entered the blood stream (directly or after first having passed part of the lymph vascular system) through some organ or some mucous membrane, without having left any demonstrable changes, or at least only changes so small as to be generally overlooked. This mode of infection I believe to be rare, but it well deserves to be considered.

2. It may be supposed that infection of the different parts of the lymph node system has taken place separately, either simultaneously or at different times. This explanation is quite certainly the correct one in a number of cases, especially where the affection is not pronounced in the different groups, where the nodes involved are not directly connected, and also in cases in which the lesions plainly vary in age; for instances, in one group encapsulated, pultaceous, and calcareous foci, in other groups recent eruption of tubercles (perhaps only microscopic). On the whole, attention, much more than formerly, must be directed to repeated infection at different times of different organs, or even of the same organ. With my experience in view I must, in this respect, quite agree with Lubarsch and Ribbert, who recently have maintained the same thing. This explanation is especially applicable in cases of coexisting tuberculosis of the cervical nodes (+ tonsils). and in the intestine with the mesenteric nodes, as well as in coexisting slight tuberculosis of cervical and thoracic nodes or of thoracic and abdominal nodes.

3. The third mode is a successive extension from a single place to the neighboring groups of lymph nodes until a greater portion of the lymph node system of the body is the seat of a tuberculosis, which, while of different age, is of about uniform appearance. This mode I consider particularly frequent and, therefore, will call special attention to it. In arriving at such a conception of a given case, however, the postmortem findings do not suffice, but an exact clinical history, with clear exposition of the main points in the development of the disease, is also essential. This is particularly true in cases of supposed primary localization in the cervical and mesenteric nodes, which, so far as my experience at present goes, very frequently shows a tendency to continued

further extension. The correctness of this will appear from the cases given, the first of which are especially instructive in regard to extension from the cervical nodes and which also (like the following cases of intestinal tuberculosis) contains instances of extension from the abdominal nodes.

With all these cases in mind there is no room for doubting the correctness of the view which I also maintained in connection with tuberculosis in children, that tuberculosis of the cervical nodes may descend to the nodes of the chest and abdomen, finally to become almost general, and, conversely, that tuberculosis of the abdominal nodes may ascend to chest and neck, and, finally, that tuberculosis of the thoracic nodes may extend upward and downward to neck and abdomen respectively, and also along the bronchi. As long ago maintained by Weigert, I consider the communications between the lymph nodes of different regions as amply sufficient to explain such extension. Observations in regard to extension of malignant tumors, especially carcinoma, and results of experiments on the transmission of tuberculosis to animals also confirm this. It is noteworthy that the infection generally seems to extend continuously, without leaps. It is another matter that we find the affection most marked in certain localities possessing numerous and large lymph nodes, *e. g.*, the upper part of the neck, the bifurcation of the trachea, the hilus of the lung, the retroperitoneal connective tissue behind the stomach, about the pancreas, etc.

The results of the findings in the cases narrated and of considerations called forth by them warrant the following conclusions:

First, that primary tuberculosis of lymph nodes is quite frequent in adults.

Second, that besides in the thorax tuberculosis is quite often primary in the abdomen and especially in the cervical nodes.

Third, that not seldom it is found generally distributed, and

Fourth, that it often extends from one place to another, during years or dozens of years, so that finally a great portion of the lymph node system has been attacked by tuberculosis.

In this connection the question of the duration and latency of such tuberculosis is raised. We must disregard the possible

occurrence of latent tubercle bacilli in the lymph nodes of adults as no special investigations have been made on that point. From the experience in children and the result of experiments concerning the occurrence of latent tubercle bacilli in adults, which have previously been related, it appears reasonable, however, that here also such infection, without demonstrable anatomical changes, might frequently be demonstrated, and that tubercle bacilli are capable of remaining latent for some time.

It is a different question how long one of the described forms of chronic tuberculosis of lymph nodes of slow, successive development may remain latent before death occurs from secondary tuberculosis of other organs, or accidentally from other causes. Recently Lubarsch[1] on the basis of personal observations has come to the conclusion that a clinical latency for 10 years or more of tuberculous foci is possible. With my observations in view I must place the limit considerably higher. The clinical data in certain of our cases point to a latency of 13–14–15 years (see Nos. 1, 3, 4, and 110, '00), of 28–29 years (No. 2, and a case not narrated), in one case to one of even longer duration.

The duration of latency in such forms of tuberculosis, therefore, may safely be placed at 20–30 years, and probably may be considerably longer. The term latency is used so as to comprise both cases of well encapsulated old tuberculous foci (virulent or non-virulent) in lymph nodes and the not less important latent chronic affections of lymph nodes in which the tuberculosis has made its way from one place to another in the course of many years. And I do not consider whether in these chronic affections of lymph nodes death is caused by complications brought about by them[2] or whether the affection remains latent the whole time and death is brought about by other causes. It is worthy of note and clearly brought out by the clinical histories that the infection in these cases of chronic tuberculosis of lymph nodes often dates far back in childhood; a history of enlarged nodes in childhood with repeated operations is continually obtained.

It has been shown in numerous clinical and pathological works

[1] *Loc. cit.*

[2] See the case described of complicating compression of the duodenum with secondary dilatation of the stomach.

(as the extensive work of C. Sternberg)[1] that the course of lymphatic tuberculosis may take on various forms. Certain cases do not take a latent course, but a peculiar clinical picture may be produced, characterized by a prolonged febrile condition and increasing anemia. Clinically such cases often will pass under the name of pseudoleukemia and the real cause of the disease will not be cleared up before the autopsy. It appears that these peculiarities are seen particularly in cases of rapid course showing at the same time marked breaking-down of the degenerated lymph nodes (very virulent and numerous bacilli). Among our cases showing a peculiar course are Nos. 4, 8, 10 (with a very peculiar and rare form of cutaneous tuberculosis), and particularly Case 7 which is a type of such febrile affections often of very uncertain diagnosis. I do not propose now to enter further into this highly interesting chapter as it is chiefly of clinical interest.

The localization to be considered next is primary tuberculosis of the intestine and the lymph nodes belonging to it. In order to get a view of the frequency of tuberculosis of the digestive tract in general I shall add the cases of primary tuberculosis of the faucial tonsils and cervical lymph nodes which have been discussed already in part.

Case 1. (98, '01.) A woman, 25 years old, had suffered for several years from severe attacks of epigastric pain and vomiting. She steadily lost in flesh and died of peritonitis. The autopsy revealed numerous large tuberculous ulcers throughout the small and large intestine, with perforation of one ulcer into the peritoneal cavity. In the mesentery and retroperitoneal tissue were fist sized clusters of swollen caseous lymph nodes. From the abdomen the enlargement and caseation could be followed upwards in the mediastinum, and farther upwards along the neck, as well as to the hilus of the lungs and outwards along the bronchi. A caseous bronchial node had perforated a small bronchus and set up a tuberculous bronchopneumonia.

In this case tuberculosis of the intestine and abdominal nodes must be considered primary.

Case 2. (192, '01.) A girl, 23 years old, was taken with dyspepsia and diarrhea in April; after an acute enteritis she had lived mainly on milk but had to stop this as it turned out that the milk was "bad" and came from a cow with diseased udder. The diarrhea persisted; she steadily grew thinner and cachectic and died October 13. The autopsy revealed a marked old

[1] *Ztschr. f. Heilkunde*, 1900, 19, p. 21.

tuberculosis of the lower ileum and of the large intestine (in the process of healing and constriction). Extensive tuberculosis of the mesenteric and retroperitoneal lymph nodes. Also a tuberculous affection of the apices of both lungs; in the left apex only a few small caseous and pultaceous foci; in the right apex a larger similar focus. The hilus and posterior mediastinal nodes were tuberculous, almond-sized, decreasing in size upwards; cervical nodes and one tonsil tuberculous.

In view of the extent and age of the intestinal affection, it seems reasonable to consider it primary.

Case 3. (35, '02.) Man, aged 35, who had suffered from dyspepsia since the age of 17 years. In February, 1900, pericarditis developed, and in the summer of the same year tuberculous peritonitis; he died February 14, 1902, greatly emaciated. The autopsy revealed large caseous tuberculous nodes along the trachea and isolated ones in the neck. Inveterate tuberculous pleuritis, pericarditis, and peritonitis. Numerous tuberculous intestinal ulcers and tuberculosis of the mesenteric nodes. Lungs uninvolved.

At all events a primary intestinal tuberculosis must have existed in this case; whether the entire lymph node affection, especially that of the tracheal and hilus nodes, was secondary or due to another (and older) tuberculous infection, cannot be decided.

Case 4. Man, 25 years old, who suffered for some years from tuberculous peritonitis. There was tuberculosis of the intestines and mesenteric nodes in addition to the peritonitis, but normal lungs.

Case 5. A woman, 37 years old, had been ailing for years, especially since her last pregnancy seven years before, and had suffered from abdominal pain and dyspeptic symptoms. She died from perforative peritonitis. The autopsy revealed old tuberculous ulcers in the small and large intestine; one of the ulcers had perforated; there were adhesions and strictures. Also unilateral tuberculous salpingitis; and an old and very extensive tuberculosis with pronounced caseous and pultaceous degeneration of the lymph nodes, viz., retroperitoneal, mesenteric (most marked), iliac, inguinal, and a continuous chain in the posterior mediastinum, along the trachea, in the pulmonary hilus and on the left side of the neck. No trace of pulmonary tuberculosis.

We have here an old primary tuberculosis of the intestines and mesenteric nodes; the affection of the thoracic nodes appears to be secondary.

Case 6. (65, '03.) A man, 37 years old, died of empyema pleurae. The autopsy revealed an old left-sided tuberculous empyema. In the apex of the right lung a few tubercles and a pultaceous focus the size of a grain of maize. Large caseous lymph nodes on both sides of the neck, in the posterior mediastinum and pulmonary hila; tuberculous ulcers in the intestines, and beginning tuberculous peritonitis.

The affection of the lymph nodes and intestine is the oldest;

that of the lung was slight, looked more recent, and was completely encapsulated. The affection of the lymph nodes must be looked upon as independent of that of the intestine (descending from the neck?).

To these six cases of primary tuberculosis of the intestinal tract must be added two which were not narrated, and eight cases of primary tuberculosis of the abdominal nodes, mainly mesenteric and retroperitoneal (in one case the nodes in the groins and mesocolon). In order to include all cases of primary infection through the digestive tract, however, it is necessary further to include the three cases of extensive lymph node disease already recorded, in which infection had occurred through the throat or intestine, or both; six of the cases of general infection where the throat was considered the place of invasion, and five cases in which was assumed a primary infection through the intestinal tract partly associated with infection through the respiratory tract. Some of the latter cases, however, were somewhat doubtful. But I will not include other and not uncommon cases of extensive lymph node tuberculosis of probable origin in the abdomen or cervical nodes, and associated with tuberculosis of other internal organs, especially the lungs, which renders difficult the analysis of any single case.

We thus altogether have about 30 cases of certain or, in a few cases, very probable primary tuberculosis of the digestive tract, *i. e.*, 5.5 per cent of the total number of autopsies or 7.7 per cent of all tuberculous cases. As also most of these cases, viz., 22, directly or indirectly led to death from tuberculosis, it is of interest to compare fatal cases of this class with the total number of deaths from tuberculosis, which was 124. The percentage then is 18. This is the highest figure reached; but several cases have been omitted in which the postmortem findings were too intricate and the data too scant to admit of any definite conclusion.

In comparison it may be stated that Hof (*loc. cit.*), in considering all autopsies on tuberculous adults, found 5.9 per cent of primary infection through the digestive tract (2.3 per cent of all adults, while in 84.9 per cent of the cases infection was through the respiratory tract. Zahn found primary tuberculosis of the

digestive tract in 2.4 per cent, v. Hansemann in 3.5 per cent By including the autopsies in children, Lubarsch (*loc. cit.*) reached 5.5 per cent, and by also including tuberculosis of the tonsils, 6.3 per cent.

Primary localizations of tuberculosis, outside the respiratory and digestive tracts and lymph nodes, are not numerous. We shall only mention that they were observed as follows:

Eleven cases of bone- and joint-tuberculosis, six of which possibly were secondary, mainly to affections of the lung.

Eight cases of genito-urinary tuberculosis (six of these probably secondary).

One case of primary tuberculosis of serous membranes.

Two cases of primary tuberculosis in spleen and pancreas.

On account of the rarity the two latter cases are related:

1) 153, '02. In a man, 46 years old, who died of diabetes mellitus, and in whom there was no trace of tuberculosis in the lungs, intestinal tract, or lymph nodes, a pea-sized, rather firm nodule was demonstrated in the pancreas, which on microscopic examination was seen to consist of conglomerate tubercles.

2) At a legal autopsy in a woman, 32 years old, who had died of carbon monoxide poisoning, the spleen was found enlarged (weight, 170 g.); throughout it were numerous pea-sized yellowish-white nodules, which consisted of degenerated tissue. Microscopically the nodules were seen to consist of fibrous and hyaline tubercles, in which a few tubercle bacilli were found. No trace of tuberculosis in the rest of the body (particularly not in the lungs, intestines, or lymph nodes).

Otherwise, in the bulk of the cases, both those of fatal tuberculosis and the much more numerous ones of latent and obsolete tuberculosis, the localization was in the lungs and thoracic nodes which probably most frequently had been primarily infected.

It now remains to consider somewhat the most common of all localizations in adults, namely, pulmonary tuberculosis, especially in its connection with other tuberculous affections, and first of all with the frequent affections of lymph nodes. When at the same time tuberculous processes are found in other internal organs and first or all in the lymph nodes, one is prone to conclude that the pulmonary affection is primary; and from sputum containing tubercle bacilli tonsils and cervical nodes as well as intestines and mesenteric nodes may be secondarily infected. The question is

whether, as a rule, this is the case, or if not, the pulmonary disease may be secondary, as we frequently had to assume in our study of tuberculosis in children.

That this really may be the case will appear from a study of the cases which have been related. It has been shown that in adults as well as in children perforation into the bronchi from tuberculous nodes may take place and set up a local pulmonary tuberculosis; further, that tuberculous nodes may become adherent to the lung tissue and thus infect it. In these cases, as well as when the lung tissue is attacked by lymphogenous infection (from infected hilus or bronchial nodes, which is not of rare occurrence), we are dealing with involvement of the lungs from neighboring tuberculous organs, mainly lymph nodes. This source of pulmonary tuberculosis is also commonly recognized, though as a rule no great importance is attached to it.

The principal question, however, is whether hematogenous infection of the lungs by tubercle bacilli from some tuberculous focus may occur (either directly or indirectly after first passing through lymphatic channels). In other words, whether pulmonary tuberculosis in adults cannot be traced back to a tuberculous affection which existed at a remote time, perhaps in childhood, and which cannot be brought into direct relationship with the pulmonary lesions in any of the ways described. Old tuberculosis of lymph nodes, first of all, would then receive attention. It is needless to say that in all such considerations the possibility of a new infection (as by inhalation) must not be lost sight of.

It is this mode of infection which in late years has given rise to much animated discussion, especially in Germany. Among pathologists, Ribbert[1] in particular has come out strongly in its favor and thereby called forth most of the other works on the subject. Of later date we have the various well-known addresses by v. Behring[2] to the effect that infection with tubercle bacilli takes place in early childhood, and that subsequent pulmonary tuberculosis is traceable to it. According to this theory, for

[1] *Ueber die Ausbreitung der Tuberculose*, Marburg, 1900; *Deutsche med. Wchnschr.*, 1902, 28, p. 301, and 1904, 30, p. 300.

[2] *Deutsche med. Wchnschr.*, 1903, 29, p. 689; 1904, 30, p. 193; *Tuberculoseentstehung, Tuberculose bekämpfung und Säuglingsernährung*, 1904.

which v. Behring himself could not furnish much evidence, the lungs in all probability must be thought to be infected by way of the blood.[1] Similar views have been brought forward for some time from clinical observations, chiefly by Volland,[2] and from statistical considerations by Antvord.[3]

Ribbert derives his arguments chiefly from a consideration of miliary tuberculosis which he finds may show every transition to ordinary pulmonary tuberculosis, and he points to the frequent presence of older tuberculous changes which it appears reasonable to connect with a fresh pulmonary tuberculosis of more recent origin. He does not, however, deny an aërogenous pulmonary tuberculosis, but claims this to be a comparatively rare form, as he cannot recognize the force of the arguments brought forward in favor of frequent inhalation of tubercle bacilli. Ribbert's hypothesis in the main has been accepted by v. Baumgarten and some others, but has also met with strong opposition, especially from Cornet, and particularly Schmorl.[4] The latter has taken the matter up in its entirety and made use of the abundant material of the Pathological Institute of Dresden. His result is that the possibility af the hematogenous origin of pulmonary tuberculosis cannot be denied, but that most cases are attributable to air-borne infection. He offers as proof the fact that the oldest lesions generally are found in the lungs (the lymph nodes may even be entirely free from tuberculosis), that the hematogenous eruption in the lungs has its principal seat not at all in the apices, and finally, that in small isolated nodules in the lungs it is often possible to demonstrate directly that the mucous membrane of the small bronchi have been primarily attacked (as had previously been shown by Birch-Hirschfeld).

In regard to this whole matter I must confine myself to accentuating certain points based on personal experience. In the first place I must agree to what has first been emphasized by Orth

[1] However, v. Behring has modified his views so much that he now to some extent seems to believe that infantile tuberculosis only creates a susceptibility to a later new infection, eventually of the lungs, and by another route, such as inhalation.

[2] *Ztschr. f. klin. Med.*, 1893, 23, p. 50; *Münch. med. Wchnschr.*, 1904, 51, p. 879.

[3] *Norsk Mag. f. Laegevid.*, 1895, 4 R, 10, p. 1013; and 1898, 4 R, 13, p. 337

[4] *Münch. med. Wchnschr.*, 1902, 49, p. 1379.

and later by Ribbert, viz., that miliary tubercles (by hematogenous infection) frequently are found to be most numerous and largest in the apices, and that, chiefly in young persons, cases occasionally are met with in which it is a matter of grave doubt whether the pulmonary tuberculosis is of aërogenous or hematogenous origin (apparent transitional forms). Likewise I must maintain, with Ribbert and Orth, that pulmonary tuberculosis of hematogenous origin in its further course conducts itself like the forms commonly considered due to inhalation.

I must further emphasize that in adults, in addition to slight, well limited, and apparently recent pulmonary tuberculosis, one also finds in other organs tuberculous changes which are extensive and plainly of older date; these are chiefly in the lymph nodes, but also elsewhere, as in bones, intestinal tract, genitalia, etc.

It is therefore natural to suppose that in such cases the lungs have been secondarily infected by way of the blood (directly or indirectly) from the older tuberculous foci, for instance in the lymph nodes. This seems the more reasonable when at the same time scattered tubercles are found in other organs which must be due to hematogenous infection. But naturally, in the given case one cannot exclude the possibility of two infections having occurred, the later by inhalation. In that case it might be supposed that the old tuberculosis of the other organs had brought about increased susceptibility to a subsequent new infection. This latter explanation appears most probable in case there is a history of lymph node tuberculosis in childhood of which no trace remains, of a healed bone tuberculosis, etc.

If, however, we are dealing with a chronic progressive lymph node tuberculosis which the clinical history also shows to have existed for years, then hematogenous infection of the lungs appears to be most plausible, especially when only small recent foci are found. If the lungs at the same time are the seat of a scar, an encapsulated caseous mass, calcareous deposits, etc., no conclusions as a rule can be drawn in regard to this point.

Considerable material elucidating this question may be gathered from my cases (see the summaries). Here I shall touch only certain points.

For instance, the clinical history in cases of tuberculosis in adults often tells us that the patient had enlarged lymph nodes in childhood. This statement was made in not less than five of the cases of general lymph node tuberculosis and in one case of tuberculosis of the retroperitoneal nodes, and is of course of the greatest importance in the determination of the point of origin. Similar statements are strikingly rare in the case of the other autopsies during this period. When at autopsy no trace of tuberculous nodes or only scars or small encapsulated caseous foci are found in the neck then it must be considered very doubtful whether the pulmonary tuberculosis which later caused death had any connection whatever with the enlarged nodes which existed in childhood.

The cause of the relative rarity of a history of enlarged nodes in childhood must largely be sought in defective anamneses. For comparison it may be stated that Dr. Frich[1] who collected and elaborated the 354 cases of pulmonary tuberculosis admitted to one of the medical wards of the hospital in the course of ten years found that in 36 cases (10.4 per cent) enlarged nodes had existed.

In cases also of primary tuberculosis of other organs than the lymph nodes small recent foci of tuberculous inflammation in the lungs were not seldom found, for instance, in some of the cases of primary tuberculosis of the intestinal tract. Here also it seems probable that the pulmonary tuberculosis was secondary and hematogenous. It will always be a question of personal judgment how much to include in this regard. On studying the records of the cases of fatal pulmonary tuberculosis I should assume that at least 15–20 per cent with greater or less probability must be looked upon as secondary, and of probable hematogenous origin; cases of primary tuberculosis of the thoracic nodes are included.

But the great mass of cases of tuberculosis of the respiratory tract, both the fatal and even more the latent and obsolete ones, do not appear to have any connection with older tuberculosis lesions in other organs so far as can be determined by the post-

[1] *Norsk Mag. f. Laegevid.*, 1904, 65, p. 782.

mortem findings and the generally scant clinical data. However, I have no doubt that this proportion of 15–20 per cent would be considerably increased if further careful investigations were made, especially clinically, with the view to establishing the previous existence of tuberculosis of lymph nodes, and not only of primary infection of the thoracic nodes but also, of what has been given too little attention, descending cervical or ascending abdominal lymph node tuberculosis.

The idea of the importance of old lymph node tuberculosis in connection with the later development of ordinary pulmonary tuberculosis also receives a support in statistical works on the frequent occurrence of tuberculous nodes in childhood and of scrofulosis in general.

In our Norwegian medical literature such a connection has been maintained by Antvord chiefly[1] who holds that in as many as two-thirds of all cases of tuberculosis infection occurred in childhood. He believes that at least one-fourth of all adult consumptives were scrofulous as children. As tuberculosis in children most frequently is found in the lungs and bronchial nodes then, according to Antvord, the original infection was most likely aërogenous. He does not say in what manner pulmonary tuberculosis should arise in adults who were scrofulous in childhood; probably, however, this must be thought to occur, at least in part, by way of the blood. He does not mention new infection in adults as an explanation, and believes the study of tuberculosis in childhood will give hope of finding the real source of infection.

As has been mentioned, Volland in several articles has advocated similar views. He also believes infection to take place in early childhood, or even infancy, and mainly through the mucous membranes of the throat and nose, and through the skin, from dirt. From the lymph nodes the tubercle bacilli enter the blood and reach the apices of the lungs, where secondary tuberculosis develops. Volland leaves a new infection out of consideration.

The articles of Laser and Randers, in which also the frequency of tuberculosis of the cervical nodes in children has been emphasized, have already been mentioned.

[1] *Loc. cit.*

The importance of a preceding scrofula seems to be still clearer brought out by experience obtained at hospitals for scrofulous patients whose further fate has been followed. This is shown by the records given by Dr. Schepellern from the hospital at Refsnæs in Denmark.[1] Of 814 discharged scrofulous children, 155, or 19 per cent, were dead 10 years after discharge, and 18 per cent died of tuberculosis. Of the remaining 81 per cent, 167, or 21 per cent, still were scrofulous, and 50 of these had developed pulmonary tuberculosis. If cure is not obtained, the condition gradually passes into consumption, and this is the more common the older the person becomes. After 10 years one-third had either died of tuberculosis or were suffering from it.

German works on lymph node tuberculosis show similar results. As an instance I may mention the work of Blos[2] on tuberculous lymphomas and their relation to pulmonary tuberculosis. It treats of the children with tuberculous lymph nodes (cervical in 89 per cent) treated in Czerny's clinic in Heidelberg. Of 160 operated on at least three years previously, 26 per cent had developed tuberculosis of the lungs and 14 per cent tuberculosis of other organs. Blos also compared similar observations from other surgical clinics. The percentage of operated scrofulous children who later developed pulmonary tuberculosis varied from 10 and 11 to 22 and 26 per cent.

These observations are substantiated by many of the cases described by me, and everything is strongly in favor of pulmonary tuberculosis being secondary (by hematogenous infection) in a not small proportion of cases.

As the result of these considerations, I must maintain that also in adults pulmonary tuberculosis may be and often is secondary. The cause is then to be sought in tuberculous foci in other organs, but mainly in the lymph nodes, and the infection of the lungs occurs most likely by way of the blood (from the thoracic nodes the infection might also easily occur through the lymphatics). I hope in the numerous cases recorded to have furnished anatomic support for such a view. With my obser-

[1] Cited from Antvord's article in 1898.

[2] *Mittheil. aus den Grenzgeb. d. Med. u. Chir.*, 1899, 4, p. 520.

vations in mind (including the latent bacilli in children), I also believe I may maintain that much more stress must be laid upon primary infection through the digestive tract, and not least on infection through the throat, than has been done formerly. Clinical experience also points in the same direction.

EXPERIMENTAL MEASLES.*

LUDVIG HEKTOEN.

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INTRODUCTION.

THE search for the cause of an infectious disease like measles becomes greatly simplified when we learn how to secure the unknown "virus" in relatively pure form unmixed with common microbes. Various methods may now be applied to the investigation of the virus. The transmission of measles from mother to fetus would seem to point to the presence of the cause of the disease in the blood. In the 20 cases of fetal measles collected by Ballantyne[1] it seemed that the infection of mother and fetus must have been simultaneous because the eruption in both corresponded in character. In order to learn something further as to the presence in the blood of the cause of measles, inoculations of human beings would seem to be necessary because, so far as we now know, this disease is probably not communicable to animals. Grünbaum's[2] experiments with measles in the chimpanzee appear to have given negative results.

In the present article I propose to review briefly the results of the inoculation of measles as they appear in the literature, and then to record two experiments of my own from which I believe certain conclusions may be drawn.

REVIEW OF THE LITERATURE.

The first attempt at inoculation of measles of which we have any record was made by Francis Home, in Edinburgh, in 1758. Although he nowhere in his writings makes any acknowledgement thereof it has been regarded as probable that he received the inspiration to make this attempt from the following suggestive statement by Alexander Monro (secundus):[3]

*Received for publication January 10, 1905.

1 *Arch. of Pediat.*, 1893, 10, p. 301; also *Manual of Antenatal Pathology and Hygiene*, 1902, p. 196.

2 *Brit. Med. Jour.*, April, 9, 1904, 1, p. 817.

3 *De venis lymphaticis valvulosis et de earum in primis origine*, 1757.

How successful inoculation of smallpox has turned out is known to all, but I regard it as altogether certain that inoculation of measles will be much more useful and successful. For it is well-known how liable this disease is to infest the lungs, and how great destruction it causes there. This seems in the first place to be due in large part to the contagion which flies about in the air, and is drawn into the lung cells in breathing, and persistently clings to them, and causes a cough there, or in other words, excites an attempt by nature to drive off the noxious matter. If measles were really to be induced by inoculation artificially produced, it is very likely that the lungs would be more free from inflammation, and in general the disease would attack the skin only. If this should turn out so what a great profit and utility it would bring to mankind! The experiment can bring about no inconvenience or loss. It is probable that inoculation can be performed, if only the pustules and spots of matter can be rubbed on cotton, and if this (either fresh or put on glass carefully covered and preserved) be applied to a little wound, exactly in the same way as variolous matter.

What Home himself appears to have hoped to accomplish by this experiment, as well as the methods he employed, may be given best in his own words:[1]

Considering how destructive this disease is, in some seasons; considering how many die, even in the mildest epidemical constitution; considering how it hurts the lungs and eyes; I thought I should do no small service to mankind, if I could render this disease more mild and safe, in the same way as the Turks have taught us to mitigate the smallpox. I suspected strongly that the cough, often so harassing in the mildest kind, was produced by receiving the infection mostly by the lungs; and I hoped that this symptom would abate considerably, if I could find a method of communicating the infection by the skin alone.

But there was no matter to be had from the measles. A woolen glove taken from the arm of a measly patient would not answer my purpose, as a part of the infection might be drawn in by the lungs. I could not find a sufficient quantity of scaly matter, after the measles were dried to serve my purpose. I then applied directly to the magazine of all epidemic diseases, the blood.

As the measly matter behoved to be but a small portion of the whole mass, I chose to make use of the blood when it contained the morbific matter in the highest state of acrimony. In that situation the blood seemed to me to be, the next day after the turn of the measles, when their matter by juxtaposition and stagnation becoming more acrid, as we know happens in all eruptive cases, was again absorbed into the mass of the blood, and was the cause of the inflammations which happened then and afterwards. I chose to take it from the most feverish patients.

I was not contented with that alone, but thought that I should get the blood more fully saturated with what I wanted, if it was taken from the cutaneous veins amongst the measles, than if I took it from a large vein, where there was a much greater proportion of blood from the more internal

[1] *Medical Facts and Experiments*, Edinburgh, 1759.

parts, than from the skin. I therefore ordered a very superficial incision to be made amongst the thickest of the measles, and the blood which came slowly away was received upon some cotton.

What I had most to fear was a deficiency of morbillious matter; and therefore, it was plain, that the sooner it was applied, and the more close it was kept, the better chance it had to succeed. An incision in each arm, as is made in the smallpox, was giving it a greater opportunity to take place. I thought it a very material point to allow the wounds to bleed for a quarter of an hour before the cotton was put in, that the fresh blood might not wash off, or too much dilute the morbillious matter. I have always let it remain three days in the wound. I have kept exactly to all these circumstances, finding that the observance of them was attended with success.

Under an uncertainty whether I was able to produce this disease, I made a trial and found it succeeded. This success encouraged me to proceed towards completing the discovery. From the prejudices of mankind, I found it difficult to get the blood as I wanted it, and much more difficult to find subjects for inoculation. I shall circumstantially narrate the experiments which are already made, and which appear to me amongst the most material that ever were made for the good of mankind, in this part of the world; for the inoculation of the smallpox was already established in Turkey before it was brought here. Even there it was probably the effect of chance, and not the result of reason. This improvement of our art has been long wished for by many, but never yet, so far as I know, been put in practice.

According to his own records Home attempted to inoculate measles in 15 different persons, and he concludes that in most instances he succeeded in producing the disease in a mild and modified form. In his "Principia Medicinae" (1770) he summarizes the matter in the following statement:

"Morbilli per insitionem, ope sanguinis infecti, communicantur, uti a me usu confirmatum est. Die sexto plerumque febricula sese monstrat, mitissima tussicula, sine insomnio et inflammationis symptomatibus, concomitante; et neque febre hectica, neque tusse, neque oculis inflammatis succendentibus."

Home's inoculations have been accepted as successful by many contemporary and later writers and his name has been handed down even into the text-books of the present as the one who first succeeded in inoculating measles. Perhaps the following letter by J. Cook, in the *Gentleman's Magazine and Historical Chronicle* for 1767, conveys a fairly accurate idea of how the question presented itself to some at least of that period:

The measles, though not so fatal as the smallpox, is yet attended in the natural way with many dangerous symptoms, and often produces very troublesome effects. I would therefore beg leave to recommend to the public the

practice of inoculation in this distemper as well as in the other, and am confident that by this method many may be preserved from that malignant sort which often proves mortal. and is always dangerous.

Dr. Francis Home was the first who attempted this practice at Edinburgh about nine years ago, since which, many physicians in that country have followed his example, though I do not find it is much encouraged in England, though in smallpox it is now become universal.

The method is easy, may be performed with safety by a careful nurse, and is not attended with the remotest danger.

Dip a little bit of cotton or lint, on the watery humour that stands in the eyes of persons ill of the measles about the time of the crisis, make a slight scratch in the arm, above the elbow, of the person to be inoculated, put the watery pledget upon the incision and cover it with a bit of sticking plaster to keep it on; and this without further trouble will produce the measles in a gentle and favorable degree, which, during the whole course, will want no other care than that of keeping the patient moderately warm, nor any attendance but that of watching the fever, and encouraging the crisis, which, in a few days will carry off the infection, and complete the cure. This epidemic disease should be communicated to those young subjects who have not yet had it, when it makes its first appearance in any neighborhood, by which the dangerous symptoms that often attend it will be effectually prevented.

We see that a different source of material for inoculation has been selected, namely, "the watery humour that stands in the eye" in measles, but I have looked in vain for other evidence than the mere statement contained in the letter that this method actually produced the disease "in a gentle and favorable degree."

One year later E. Spry[1] writes:

The method of procedure in inoculating for measles does not differ from that which, as we have before described, is to be followed in the case of smallpox. I think there is only one thing which in this case deserves notice as being peculiar, viz., that the linen threads which are used for introducing the contagion ought to be impregnated with blood, for matter is rarely found, drawn from the pustules of the measles near the tip or a little way from it.

In this method of treatment, not yet so common as the earlier one all the symptoms are found to be less serious—a fact of which I am quite certain, not from one observation, but from the study of many cases.

But unfortunately Spry was also content with merely making the bare statement quoted, so that we have no actual facts to aid us in forming an independent opinion as to the nature of the disease he claims to have produced.

Home's inoculations were regarded as highly successful by Buchner, Nils Rosén von Rosenstein,[2] Holst[3] and others and the

[1] *De variolis ac morbillis iisque inoculandis*, 1768.

[2] *Kinderkrankheiten*, 1798, p. 352. [3] *Med. Chir. Ztg.* 1811, p. 205.

practical use of the method, or some modification thereof, was urged. According to Themmen desquamated skin, blood, tears, nasal discharge, and saliva were recommended by various writers for inoculation, the application to be made to each arm, the skin either being left intact or cut.

Judging from the following quotation Erasmus Darwin[1] was either not familiar or not favorably impressed with Home's work:

> . . . . it is probable that inoculation might disarm the measles as much as the small pox, by preventing the catarrh, and frequent pulmonary inflammation, which attends this disease; both of which are probably the consequence of the immediate application of the contagious miasmata to these membranes. Some attempts have been made, but a difficulty seems to arise in giving the disease; the blood, I conjecture, would not infect, nor the tears; perhaps the mucous discharge from the nostrils might succeed; or a drop of warm water put on the eruptions, and scraped off again with the edge of a lancet; or if moistened with a little warm water? Further experiments of this kind would be worthy the public attention.

Robert Thomas[2] says that notwithstanding Home's success, "inoculation for the measles is seldom or never practiced. The few who have been induced to attempt it, have not, I believe, made quite so favorable a report of it; on the contrary, it has been said to produce an aggravated disease." And Ronalds[3] writes that Home's plan "has been adopted since his time upon a small scale apparently without furnishing the desired results, but it certainly appears worthy of a more extended trial."

The first and practically the only serious criticism of Home's experiments and his interpretation of the results was made by Thomassen a Thuessink[4] and his pupil C. J. Themmen[5] in Holland. Themmen in particular calls attention to the possible danger of unrestricted inoculation of measles if the inoculation really transfers the disease; indeed Girtanner is said to have warned against inoculation because it was sometimes followed by serious pulmonary and other affections. The conveyance of measles by the blood they consider as remarkable, to say the

[1] *Zoonomia*, 1796, 2, p. 243.

[2] *The Modern Practice of Physic*, third Am. from fourth Eng. ed., 1815, p. 223.

[3] *Lond. Med. and Surg. Jour.*, 1816, 36, p. 13.

[4] *Ueber die Masern*. 1831. p. 231.

[5] *Dissertatio Medica Inauguralis Historiam Epidemiae Morbillosae, Groningae, Anno* 1816, *Observatae, Exhibens*, 1817.

least, in view of the fact that Hoffmann had found that in small pox the blood was not infectious.

Themmen also doubts that measles really was transferred by Home's inoculations because the symptoms appeared so early as on the sixth day, whereas Van den Bosch had observed that measles which came in the natural way broke out on the 14th day after exposure—subsequently established as the rule chiefly by the observations of Panum[1] in the Faroe Islands. Inasmuch as the inoculations were made at the time when the disease was endemic, he considers it likely that the disease had been acquired in the natural way previous to inoculation. This opinion was greatly strengthened by the wholly negative results of Themmen's own experiments. He placed blood of measles patients, taken at the height of the exanthem upon small wounds on the arms of two ch...iren; cotton saturated with the tears of a measles patient upon a ruptured vesicle on the arm of an infant; in another case a similar experiment was made with cotton soaked in the perspiration of a patient thickly covered with the eruption of measles; and in the fifth experiment he placed cotton soaked in the tears of a patient with measles upon the intact skin of each arm of a girl. "Though all these things were performed cautiously and in accordance with the precepts of the authorities, yet we saw no effects therefrom, and these five children, although they had not previously been attacked with measles, remained entirely free from this disease," says Themmen, who acknowledges, however, that the children were apparently not very susceptible to measles because they all lived in houses in which measles was prevalent and yet remained free from the disease.

But the Dutch authors are not content with this open attack upon Home's experiments. Thomassen a Thuessink indicates that there may be reason to doubt that the experiments were ever made. He says that "many doubt that these inoculations really were made by Home in the year 1758, as old physicians who then lived with Home, in Edinburgh, like Professor Black, Cullen,[2]

[1] VIRCHOW's *Archiv*, 1847, 1, p. 492. (In this classical article it is established that measles is most contagious in the period of early efflorescence and that 14 days elapse between exposure and appearance of eruption).

[2] Cullen makes no mention of inoculation of measles in the early editions of his *First Lines of the Practice of Physic*. But Themmen (*loc. cit.*) quotes Cullen (*Anfangsgr. der*

Duncan, and Gregory told me that they knew nothing of the experiments." Thomassen a Thuessink visited Edinburgh in 1784 and 1785, walked the hospitals with Home himself, and he says that he "often did not see the results which Home indicated to us." Themmen finds it rather peculiar, too, that Home "who praised the usefulness of this inoculation so highly, afterwards undertook no further experiments in this matter."

Very unlike so many others who have made experimental inoculations with measles, Home left behind him full records of his experiments. Inasmuch as the doubts expressed by the Dutch investigators as to their genuineness have not been and probably will not be more fully substantiated than indicated in the foregoing, it may not be without interest to try to determine the possible value of Home's experiments upon the basis of the facts given in his own records. These facts are summarized in Table I.

Examination of this table cannot but lead to the conclusion that probably not a single one of the 15 cases inoculated by Home had measles as a result of the inoculation. In support of this view I may point out that in no single case is the period between inoculation and the appearance of the rash given as more than 10 days, but generally as less, and even so short as seven days, whereas we now know definitely that the period between exposure and eruption in measles is 13 to 14 days.

If any of Home's cases really had measles, which seems quite doubtful indeed from his descriptions, then we are without information as to what steps he had taken to exclude infection by natural routes before making his inoculations. Lack of confidence in his diagnosis only increases when we read that case 10, which is described as a typical case of measles from inoculation, "took measles again" a few weeks later. On the whole there seems to be no escape from the conclusion that Home's claim to have produced measles by inoculation is without foundation.

In the meantime other experiments had been made. Thus Pansonia of Istria is mentioned by Themmen and Thomassen a

Pract. Arzneyk, Leipz., [1789] 2, p. 94) as having "declared in his lectures that the effects of inoculation were dubious, since, out of 12 infants, only one was afflicted with measles when inoculation was performed, and concerning this one it was by no means evident at the time, whether the measles which came upon him were really to be ascribed to inoculation, or rather to contagion received somewhere else."

TABLE I.

HOME'S FIFTEEN CASES OF INOCULATION OF MEASLES.

No.	Nature of Material Inoculated	Age of Person Inoculated	Date of Inoculation	Date of Appearance and Nature of Symptoms	Date of Appearance and Character of Eruption	Remarks
1	"Blood taken from a measly child two days before."	7 months.	March 21.	March 27: Hot, sneezing, eyes watery, no cough.	March 29: Three pustules on face and one on back. March 30: "About a dozen out." April 1: "A few more measles." April 2: "A few more out on face."	This subject had "a scabby head," running behind the ears, and an eruption over its body" before it was inoculated.
2	Same as in 1, but kept ten days loosely in pocket book.	8 years.	March 27.	"The sixth day this child sneezed much, but never was hot or struck out."		
3	Blood one day old.	6 years.	April 20.	April 27: Hot, restless, sneezing. April 28: "A great quantity of water comes out of her eyes."	April 30: "Has had the measles out since yesterday." May 2: Measles gone.	Sisters.
4	Same as in 3.	3 years.	April 20.	April 27: Hot, sneezing.	April 30: "Some measles out." May 2: "Measles pretty large."	
5	Same blood as in 3 and 4, but 14 days old. Carefully kept in a glass.	8 years.	May 3.	May 10: Uneasy. May 11: Running at eyes, sneezing.	May 12: Some spots present. May 13: "About two dozen out." May 15: All measles gone.	
6	Same as in 5.	8 months.	May 3.	May 10: Hot, sneezing, running at eyes, coughing.	May 13: "Three dozen measles appeared."	
7	Same as in 5, kept five weeks.	8 years.		No symptoms.		
8	Blood seven days old.	13 years.	June 3.	June 9: Hot, sneezing, and a little cough.	No eruption.	Measles 2 years previously.
9	Same as in 8, but ten days older.	5 years.	June 14.	June 18: Shivering, headache, sneezing.	June 21: "Measles beginning to appear." June 22: More measles June 23: "Still out." June 24: "Almost all gone."	
10	Mixture of blood taken May 27 and June 27.	18 months.	July 6.	July 9: Feverish. July 14: Coughing, sneezing.	July 14: "Had many spots out this morning but almost all gone in again." July 15: "A great many spots out, but especially on sides and thighs, where they almost touch one another." July 17: Spots disappearing.	Took measles again Aug. 20.
11	Blood two days old from 10, Aug. 27.	8 months.	Aug. 29.	Sept. 7: Hot, restless for some nights.	Sept. 7: Some spots seen yesterday. Sept. 8: "About a dozen and a half of spots." Sept. 9: Spots almost gone.	
12	Same blood as in 11.	18 months.	Aug. 30.	Sept. 7: Hot, coughing, sneezing.	Sept. 8: "About three dozen spots to be seen."	No natural measles at this time.
13, 14, and 15	Cotton with nasal discharge of measly patient on fourth day of eruption and cotton with blood.	Placed in nose of three persons (two, nasal discharge; one, blood)—no result.				

Thuessink as having made inoculations, but the latter remarks that there is no authoritative report of this work. Willan[1] inoculated three children with the fluid of miliary vesicles in measles but without success. And Chapman[2] in Philadelphia in 1801 tried in vain to inoculate measles by means of blood, tears, "the mucus of the nostrils and bronchia, the eruptive matter in the cuticle, properly moistened." On this account Dewes thought that the contagious nature of measles could be fairly disputed.

James Stewart[3] mentions the following experiment: "On the authority of the late Colonel Green, it is confidently stated that his relative, Dr. Green, of Greenwich, R. I., inoculated in the year 1799, three young persons in his circle, with blood taken from the eruptive surface of a patient laboring under an aggravated form of measles; and that these cases of inoculation were entirely successful, so that the distinctive characters were recognized by all who saw them."

Mr. Wachsel's experiment on Richard Brookes, a lad of 18, reported by Willan (*loc. cit.*) is stated by Hugh Thompson and others to have been successful, but this is, to say the very least, exceedingly doubtful. The boy was inoculated January 6, 1810, with cowpox and with fluid taken from measly vesicles. The cowpock was fully developed on the 15th. On the 22d, coughing, sneezing, and running at the eyes set in with chills followed by measly eruption on the 28th—22 days after inoculation. In the light of our present knowledge the measles in this case must be ascribed to a natural infection received about eight days after the inoculation.

In 1822 Speranza of Mantua caused inoculation of measles to be made with results regarded by him as eminently successful and so accepted without reserve by several subsequent writers. Speranza describes these inoculations as follows:[4]

. . . . we invited to perform the operation Dr. Frigori, staff physician of the Workhouse and Convalescent Hospital, where measles was always prev-

[1] *On Cutaneous Diseases*, 1809, 1, p. 106, footnote.

[2] MS. lectures cited by DEWES, *A Treatise on the Physical and Medical Treatment of Children*, 11th ed., 1858, p. 439.

[3] *A Practical Treatise on the Diseases of Children*, 1844, p. 416.

[4] *Storia del morbillo epidemico della provincia di Mantova, nell' anno 1882. Arguito un giuduzio med.—legale sopra imputazione d'Infanticidio.* Parma, 1824.

alent among the children. A slight incision was made with the lancet upon a group of the more inflamed disease-spots, and with the point of the instrument charged with the bloody matter several incisions were made on the arm of a healthy person, the wounds being covered at once with a bandage. This operation was performed, with the greatest care and under our observation, upon six boys of different ages. The boys complained, a few days afterwards, of not feeling well; about the fifth or sixth days there appeared very slight traces of cold in the head, with cough and watery eyes, which remained after the appearance of a few exanthematic spots; there was very slight febrile irritation, in some cases a mild diarrhea, and by the ninth or the tenth day after the inoculation the measles had run its course without leaving any trace of secondary malady. Dr. Frigori, not content with this result, to which he had given close and daily observation, tried the experiment upon himself;[1] the outcome was the same, but still milder, the morbid phenomena being merely a passing catarrhal affection, involving the frontal sinuses, and the pituitary membrane rather than the trachea and bronchi. A similar inoculation performed by Dr. Negri upon two boys had the result, as did our own experiments upon four other individuals, carried out in the same way. We were not equally fortunate when following the practice of Home, of Horst, and of Ronalds; that is in saturating a little cotton with the blood from an incision upon a group of exanthematic spots, and applying it to the arm before any puncture had been made. This was attempted in two cases, but the experiment did not fulfil our wishes; no catarrhal phenomena and no exanthematic spots appeared.

Speranza also states that—

In the year 1806, during the prevalence of an epidemic of measles in Parma, Dr. Rasori, staff physician of the Hospital, inoculated one of his nephews with the disease by introducing with a needle, bloody matter taken from the exanthematic sores of an infected person. The formation of papillae at the point of inoculation, with slight traces of catarrhal irritation, and immunity from the epidemic then general, were the result of this salutary operation.

From the description given by Speranza of the symptoms in the inoculated persons it would seem very doubtful, indeed, if any of them really had measles. And if the symptoms described be accepted as those of "a mild and morbillious affection," how may natural infection be excluded when we are told that measles was always prevalent among the children in the hospital and when the incubation period is given as five to six days? Under these circumstances I cannot see how it is possible to read any value into Speranza's experiments.

[1] Several writers, e. g. EBERLE (*Treatise on the Practice of Medicine*, 1830), THOMAS in ZIEMSEN'S *Cyclopedia*, and others speak of this experiment as having been made on Speranza himself.

Several older writers (Good,[1] Jörg,[2] and others) mention that inoculation sometimes produced attacks of measles quite as severe as the natural disease.

In 1834, Albers[3] without success inoculated four persons using Home's method in two, and the method of vaccination in two, the blood being taken on the second day of the eruption. From this he concludes that the blood does not contain the contagion of measles. He quotes Alexander Monro, Bourgois and Spray (Spry?) as having made unsuccessful inoculations with saliva, tears, and cutaneous scales, but no references are given.

The largest series of inoculations of measles is that of Katona (1842) in Hungary who during an epidemic made 1122 inoculations with positive results in 93 per cent. and without any evil effects. His report is very brief and without any details.[4] He inoculated as in vaccination by means of a needle dipped into fluid mixed with blood obtained by opening the little vesicles when the rash was at its height. Slight and evanescent local changes developed and on the seventh day fever appeared with the usual early symptoms of measles followed by eruption on the ninth, or at the latest on the 10th day. The symptoms were milder than usual. In two instances the rash did not develop until the 13th day. The epidemic then raging was severe according to Katona.

Here again we have the abnormally short incubation period encountered so commonly in the reports of inoculation of measles, beginning with Home, and Katona gives us absolutely no hint as to the measures used, if any were used, to determine that the disease actually was inoculated and not perchance acquired in the natural way. It is to be noted that the inoculations were made *during an epidemic* of measles so that the opportunity for natural infection was present. So far as I know we have no means by which to determine whether the percentage of inoculated that took measles was larger than the percentage that fell sick among the uninoculated. We do not know whether the communities

[1] *The Study of Medicine*, VIII, 4th Am. edition, 1826, 8, p. 34.

[2] *Handb. d. Kinderkrankh.*, 1836, p. 895.

[3] *Jour. d. Chir. u. Augenh.*, 1834, 21, p. 541.

[4] *Osterreichische med. Wchnschr.*, 1842, p. 1.

concerned at this time contained a large number of susceptible persons by virtue of having long been free from measles. Hence Katona's imposing number of inoculations can have little or no real significance in this discussion.

Bell and Stokes[1] quote with silent approval the following statement from Chapman:[2] "Not unlikely, in the instance of alleged success by inoculation (of measles), the individuals had been previously exposed to the infection of the disease, and to this mode may its production be properly ascribed; the coincidence being mistaken for the effect, one of the most common sources of vitiation in our medical inductions."

In 1850 McGirr[3] of Chicago made a series of inoculations with measles upon children in an orphan asylum in which the disease was then present. "Early in December the first case of measles was brought into the female asylum. I proceeded to inoculate from this case when the eruption was at its height. Blood was drawn from a vivid exanthematous patch on the diseased child's arm, and inserted into the arms of . . . . three children. . . . . On the fourth, sixth, and seventh days, after the inoculation, the measles appeared, pursuing a regular and typical course."

Encouraged by this result McGirr continued his inoculations, and he states that "the cases of all those inoculated, commencing from the fourth to the ninth day after inoculating, proceeded regularly with the ordinary symptoms of simple measles to convalescence which was speedy and complete. . . . ." Compared with those not inoculated the inoculated cases were much milder, and McGirr concludes that if there is no advantage in inoculation the result of this comparison would be a strange anomaly. Be this as it may, the fact that McGirr's experiments were made in an asylum infected with measles robs them wholly of weight. The incubation periods given by McGirr indicate too, that the infection in most cases was received before inoculation.

The reports of F. Mayr[4]—a name well known in the history

[1] *Lectures on the Theory and Practice of Physic*, 1848, 4th ed., p. 881.

[2] *Lectures on the More Important Eruptive Fevers, Hemorrhages and Dropsies, and on Gout and Rheumatism*, 1844, p. 118.

[3] *Northwest. Med. and Surg. Jour.*, 1850-51, 7, p. 434.

[4] *Ztschr. d. k. k. Gesellsch. d. Aerzte zu Wien*, 1852, 1, p. 6.

of pediatrics—concerning the epidemics of measles in Vienna during 1845–51 show him to have been a keen and critical observer. Although he gives only very few details of his experiments there is nevertheless good reason for regarding his results as reliable. Mayr "vaccinated" successfully six times using for inoculation the material obtained from scratching the center of a rubeolous spot. It cannot be said, therefore, that he used blood only as he himself claims, but rather blood, tissue juice, and epidermal débris mixed. In three experiments he used fresh nasal mucus which was placed upon the membrane of the nose. In two of the cases measles appeared in regular time, the eruption coming out on the 14th day. In all these instances the attacks were typical though mild, and the patients remained free from subsequent attacks. Mayr emphasizes that inoculation transfers the whole process from one individual to another and consequently offers no conspicuous advantages nor protection against complications or sequelae. There were in his cases no changes at the point of inoculation and he concludes that so long as it is not possible to localize the process as in vaccination inoculation of measles has no practical importance.

Since this time—1850—inoculation of measles has received little notice. Bufalini[1] in a report on an epidemic of measles in Siena in 1869 states that 15 years previously he tried inoculation in six cases. "Four of these had the eruption while in the other two there was no result." In one of the failures epidermal scales were used, and the "maximum effect" is said to have been obtained with the combined methods of Home and Speranza. On account of the absence of details Bufalini's experiments add nothing to our knowledge of the cause of measles.

Various writers speak of experiments with measles by Locatelli, Rossi (Rasori?), Frigeri (Frigori?), Horst (Holst?), Percival, Giotanna (Girtanner?), Vaidy, Fellegen (Tellegen?), and others, but I have not found any definite references to any articles on the subject by these persons. Undoubtedly some of the names were misspelled and have been so handed on from writer to writer.

[1] *Rev. sci. d. R. acad. di Fisiocritici*, 1869, 1, p. 111. Also Abstract by Ullersberger in *Jahrb. f. Kinderkr.*, 1871, 56, p. 282.

Reese[1] recommends inoculation in measles as the best means to prevent serious results and sequelae, but he gives no new facts and no original observations.

Hugh Thompson[2] in Glasgow accepts the inoculations of Home Wachsel, Speranza, and Katona as successful. He regards the practicability and the safety of inoculation in measles, as well as its production of a much milder attack than the spontaneous, as definitely established, and recommends that the method employed be superficial scarification followed by the application of the fluid from blisters on the skin of measles patients. In two instances however, in which Thompson practiced this method his inoculations failed.

From this review we learn that, almost without exception the recorded experiments in the inoculation of measles, for which positive results have been claimed, are without real significance. The claims that the experiments of Home, of Wachsel, of Speranza, of Katona, of McGirr, of Bufalini gave definitely positive results do not stand close scrutiny in the light of the evidence at hand: In many instances the rubeolous nature of the sickness, sometimes very mild, following the inoculation and regarded by the experimenters as measles, is not at all securely established, and in practically all cases the possibility of natural infection has not been excluded. These experiments, practically all of which were undertaken with the idea of producing a modified form of the disease, consequently permit no conclusion as to the infectiousness of the blood or other substances in measles. If we accept Mayr's results as they are given by him it may be concluded that in measles, nasal mucus and cutaneous scrapings (containing blood, epithelial débris, and tissue juices) may contain the cause of measles at or near the height of the eruption.

It already has been pointed out that in congenital measles the indications point to the simultaneous infection of mother and fetus.

In the following experiments I have tried to determine whether or not in measles at the height of the attack the blood contains the cause of the disease.

[1] *Trans. Med. Assn. Alabama*, 1880, 33, p. 396.

[2] *Glasgow Med. Jour.*, 1890, 33, p. 428.

PERSONAL EXPERIMENTS.

In these experiments special care has been taken to exclude natural infection.

1. The blood injected was taken from a boy of nine who in the later stages of desquamation after an uncomplicated attack of scarlet fever developed a rather mild but typical attack of measles. The first symptoms of measles appeared after he had been free from fever for about two weeks. There was headache, coryza, cough, running of the eyes, and mild febrile symptoms. Three days later a papular eruption was noted and on the fourth day a typical rubeolous rash was present, that soon began to fade and was followed by typical branny desquamation.

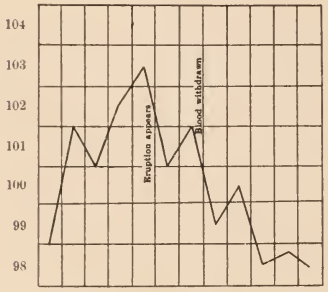

CHART 1.—Temperature curve of patient furnishing blood for Experiment 1.

On the fourth day (see Chart I) four c.c. of blood were withdrawn from the vein at the right elbow after carefully scrubbing the skin with soap and water followed with alcohol. Two flasks with ascites broth 50 c.c. (peptone broth two parts, ascitic fluid heated to 55° C. for 54 minutes one part) were inoculated[1] at once with one and three c.c. of blood respectively and placed in the incubator at 37° C. for 24 hours. At the end of this time both flasks appeared sterile, the corpuscles having settled, the supernatant fluid being clear. Subcultures made at this time upon ascites agar, glycerin agar, and Loeffler's serum and kept under aërobic and anaërobic conditions remained sterile; and the contents of the flask of ascites-broth containing one c.c. of blood remained permanently sterile.

Four c.c. of the flask of 50 c.c. of ascites-broth mixed with three c.c. of blood and kept in the incubator at 37° C for 24 hours were injected under the skin of the chest of a healthy medical student 24 years old, just finishing desquamation after an uncomplicated attack of scarlet fever, and who readily gave his consent to the

[1] In both experiments a few drops of blood were allowed to run out before inoculating the ascites-broth which was done without the needle of the syringe touching the culture fluid.

experiment. This man was not in the same hospital as the boy furnishing the blood for injection, but had been for 26 days in a different institution, at that time as well as before and afterwards entirely free from measles.[1] So far as could be learned, and careful inquiry was made, the man injected had not had any disease at all resembling measles except scarlet fever. At no time did any local symptoms appear at the site of the injection. On the 13th day after injection the temperature was 101° F; the next morning it rose to 103 (see Chart II). At nine the following morning he was given a warm bath and immediately afterwards a

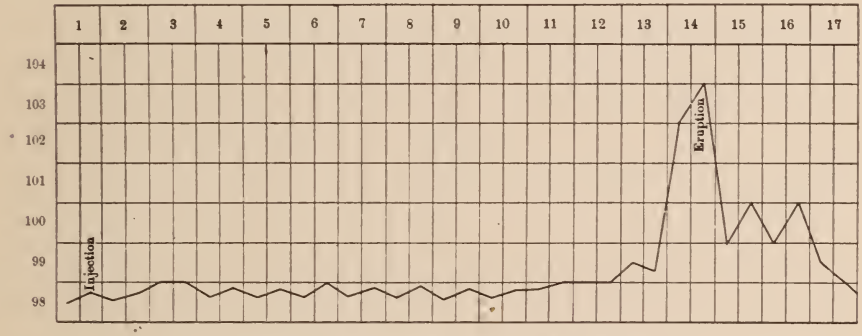

CHART 2.—Temperature curve in Experiment 1.

red, papular, blotchy eruption broke out on the forehead and spread quite rapidly to the face, neck and chest. Dr. James B. Herrick who saw him at this time felt no hesitancy in making the diagnosis of measles. By two o'clock an unmistakably typical full-blown, rubeolous rash was present over the greater part of the body. The temperature remained above normal for two days, when it fell to normal about the same time that the eruption began to fade. An uneventful recovery promptly followed without any complications whatsoever, the desquamation being branny. There was during the entire illness freedom from respiratory symptoms of all kinds. Even during the pre-eruptive period there were no special local symptoms (morbilli sine catarrho). The patient's

[1] In both experiments the injections were made by me. At the time the injections were made I had not seen any cases of measles within 24 hours. When in the measles ward the usual precautions were used and of course similar precautions were followed when visiting the subjects of the experiments — clean long gowns, caps, clean hands, etc. Freshly autoclaved syringes were used for the injections.

subjective condition was not much changed if at all at any time during his illness. The appetite continued unimpaired.

2. In this case the blood was furnished by a well-developed Irish servant girl, 21 years old, who passed through an uncomplicated attack of typical measles (Chart III). About 30 hours after the earliest appearance of the rash, which still was coming out upon the extremities, 10 c.c. of blood were withdrawn from a vein at the elbow and distributed equally among four flasks each containing 50 c.c. of broth and 25 c.c. of ascites fluid. These flasks all remained perfectly sterile so far as bacteria demonstrable by the usual methods are concerned.

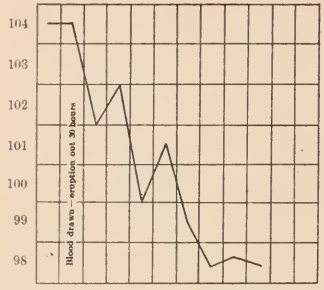

CHART 3.—Part of temperature curve of patient furnishing blood for Experiment 2.

After 24 hours at 37° C. five c.c. of the mixture of blood in ascites-broth were injected subcutaneously in the back of M., aged 28, who had not had measles so far as he knew and consented to the experiment. This patient was also recovering from a mild attack of scarlet fever and had been at the time of inoculation for 24 days the sole occupant of the isolation

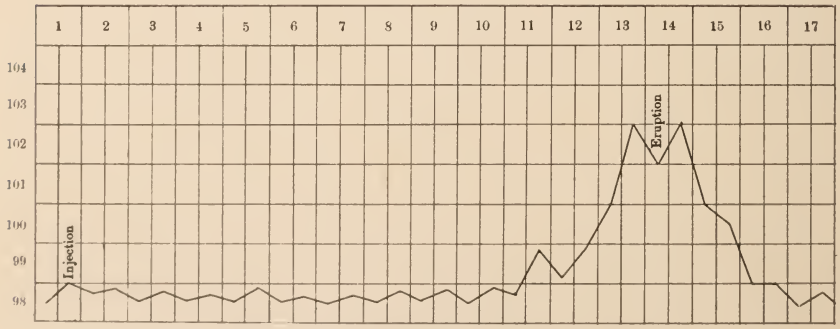

CHART 4.—Temperature curve in Experiment 2.

room of a general hospital in which at that time there were no other cases of measles. There were no local changes at the site of the injection. The temperature and general condition remained

normal until the evening of the 11th day when the temperature rose to 99.8° F. and the next day a mild conjunctivitis already suspected a day or so previously became definitely apparent. On the 13th day there was some cough, the tonsils were bright and red, and there was an increased amount of mucus in the throat. In the afternoon the temperature which was rising, reached 103° F. (Chart IV). During the next night a typical rubeolous eruption came out, the first spots being noticed on the nose and then on the forehead, face, scalp, chest, back and abdomen. The rash consisted of pink macules and papules which disappeared readily on pressure, being largest and brightest red over the face. The forehead was quite uniformly red. The patient was not seriously ill; there was some loss of appetite, but he slept well during the night, having been somewhat restless the preceding night. Recovery was prompt.

Cultures of the blood on the 13th day (one c.c. of blood in each of three flasks each containing 50 c.c. of broth and 25 c.c. of ascites fluid) remained permanently sterile.

CONCLUSIONS.

The results of these two experiments permit the conclusion that the virus of measles is present in the blood of patients with typical measles sometime at least during the first 30 hours of the eruption; furthermore that the virus retains its virulence for at least 24 hours when such blood is inoculated into ascites-broth and kept at 37° C. This demonstration shows that it is not difficult to obtain the virus of measles unmixed with other microbes and in such form that it may be studied by various methods.

ON THE TRYPANOSOMES OF BIRDS.*†

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INTRODUCTION.

THE problem of the cultivation of the malarial parasites, obviously one of great importance, is as yet untouched. The appearance of Schaudinn's remarkable paper[85]‡ in the early part of 1904 seemed to assure its definite solution. For, if the conclusions arrived at by this eminent investigator were found to be correct, all that would be necessary would be to cultivate the trypanosome stages by means of the method which has proved so successful with *Tr. Lewisi*, *Tr. Brucei*, and *Tr. Evansi*, and the problem would be solved.

It is but fair to say that we entered upon this work with the fullest confidence in the correctness of the conclusions reached by

* Received for publication January 8, 1905.

† This paper will be followed, in the next number of this *Journal*, by a second in which the other hematozoa of birds will be considered. A summary of the main results arrived at was presented before the International Congress of Arts and Science at St. Louis, September 23, and before the Society of Experimental Biology and Medicine, October 19, and was published in *American Medicine*, November 26.

‡ The references to the literature will be given in the paper "On the Hematozoa of Birds."

Schaudinn. So much so was this the case that our early results were interpreted at first as fully upholding his views. As the work progressed, however, it was found that the results obtained, instead of supporting, actually contradicted his conclusions.

Schaudinn's extensive memoir must be read in the original by those who are interested in the subject. The following summary will, however, serve to bring out the main points in his paper.

On allowing the common mosquito (*Culex pipiens*) to feed upon the blood of owls infected with *halteridium* (*Hæmoproteus noctuæ*) he found in the intestines of about 10 per cent of these insects large numbers of trypanosomes which he considered as stages in the life-history of this hemocytozoön. The oökinetes, resulting from the fertilization of macrogametes, showed from the start evidence of sexual differentiation. Thus, one type changed to an indifferent or asexual trypanosome, which then rapidly multiplied by longitudinal division, that is, in the same manner as do ordinary trypanosomes. Another type of oökinete gave rise to a female trypanosome which apparently was incapable of multiplying by longitudinal division, but by parthenogenesis regenerated all three kinds of trypanosomes. The third type of oökinete gave rise directly to eight male trypanosomes or microgametes, which, as such, are incapable of further development or multiplication, and merely serve to fertilize the macrogametes. This type of oökinete therefore corresponds to the free microgametocyte of the blood. All three forms of trypanosomes under unfavorable conditions agglutinate, forming rosettes with centrally directed flagella.

The indifferent trypanosomes, as a result of rapid multiplication, become very small, and when injected into the blood of an owl attach themselves by the flagellar end to the erythrocytes. The flagellum is then absorbed and the parasite appears as the well-known early stage of the *halteridium*. Usually during the night, the parasite leaves the surface of the red blood cell, at first, as a gregarine-like vermiculus which later takes on the trypanosome form. This, after a period of motion, again attaches itself to a cell and passes into a condition of rest, during which it grows until the next night when it again leaves its host. This alternation of the motile and rest periods continues until the sixth day when the *halteridium* has reached its full size, whereupon it leaves the cell and by rapid, consecutive, longitudinal division gives rise to small flagellates which then begin a new six-day cycle. The indifferent trypanosome in this way gives rise to the male and female *halteridia* of the blood.

The female trypanosomes when introduced into the blood likewise attach themselves to erythrocytes, but change their host-cells less often than do the former. When they leave the cell they do not take on the trypanosome form, but remain as gregarine-like vermiculi, which after a while enter new cells. The fully developed form appears as the typical macrogamete which no longer is able to leave the cell.

The male trypanosome or microgamete, when introduced into the blood, dies out rapidly. The male and female types result from and are constantly renewed by the differentiation of the indifferent trypanosomes or *halteridia*.

The results obtained by Schaudinn with the "leucocytozoön" of Danilewsky (*Hæmamœba Ziemanni*) were much the same as those mentioned above for the *halteridium*. When the blood of the owl which contains this parasite is sucked up by the mosquito (*Culex pipiens*) fertilization of the sexual elements occurs in the gut of the mosquito in the same way as under a cover-glass. The female cell or macrogamete ripens and becomes free; the microgametes are given off from the male cell or microgametocyte and fertilize the female cell. A large oökinete results which then grows in length — giving rise to a complicated coil. At the same time the original nucleus divides in rapid succession, and thus arise a large number of nuclei each of which becomes surrounded by a dense plasma zone. These small cell-territories, as in the case of the oökinete of the *halteridium*, develop into trypanosomes which become indifferent, male or female in type, according to the character of the original oökinete. These trypanosomes eventually swarm out of the large coils or differentiated oökinetes.

The indifferent forms have all the structure of typical trypanosomes. They multiply by longitudinal division and with such rapidity that the resulting young cells become extremely small: so small indeed as to be scarcely visible. Schaudinn expressed the belief that these small forms could readily pass through a Chamberland filter. When a cell divides, the two daughter cells remain attached by the posterior ends. Another feature to which Schaudinn calls special attention is agglutination. The resulting clumps are made up of indifferent cells which come together by their posterior ends, and hence their flagella are on the outside of the rosette, whereas in the case of the *halteridium* trypanosome the whips are directed centrally. It is because of this fact that Schaudinn designates this type as a "*spirochete*."

The female spirochetes are larger than the indifferent forms; the plasma is darker, the nucleus and blepharoplast are relatively small, and the undulating membrane is not prolonged as a free flagellum. The male spirochetes are so small as to be scarcely visible.

The sexual forms develop from the indifferent types. Owing to their rapid growth they soon become larger than their host-cells, and are therefore unable to enter these.

Such a spirochete, therefore, attaches itself by its posterior end to an erythrocyte which it then draws into itself. After it digests the plasma the nucleus is pushed off to one side as an elongated halter-shaped body, eventually to be thrown away as waste. This very novel view of the structure of the leucocytozoön is at variance with our own observations.

It is worthy of note that Schaudinn regards the spirochetes of recurrent fever and of geese as essentially the same as the preceding. There can be but little doubt that these organisms will be shown to be trypanosomes and hence unrelated to the bacteria.

It is evident from Schaudinn's work that he holds that such trypanosomes as chance to be present in the blood of birds represent not distinct species of organisms but merely stages in the development of intracellular parasites. This radical view, how-

ever, cannot be substantiated, for it will be shown that trypanosomatic infection is very common among birds, and that it bears no relation to the intracellular parasites which may or may not be present. Moreover, his two types of trypanosome and spirochete are really two forms of one species.

The reason for this difference in the conclusions arrived at by Schaudinn and ourselves lies clearly in the methods employed. There can be no question as to the fact that Schaudinn cultivated trypanosomes, *in vivo*, in the mosquito, but since this insect can be infected by several malarial parasites it follows that he really worked with *mixed cultures*, if we may thus use this term. The apparently positive results obtained by the injections of suspensions of the infected mosquitoes must be considered as due, not to the trypanosomes which chanced to be present, but to the unrecognized developmental forms of the hemocytozoa.

On the other hand, we cultivated the trypanosomes *in vitro*, under which conditions the trypanosomes multiply with great readiness, whereas the hemocytozoa die out. We thus obtained from a series of birds a large number of *pure cultures* of trypanosomes, representing several species. With such material we were unable to infect birds so as to give rise to intracellular parasites. As in the case of bacteria, the pure culture is after all the means that enables one to solve the relation of a protozoön to a certain phenomenon, which, by assumption, it may be credited to possess.

Occurrence of Trypanosomes in Birds.

The earliest observation on the presence of protozoa in birds is perhaps that of Gros,[34] who described organisms in the blood of the crane, crow, and fern-owl. His flagellates were 10–15 μ long, and very narrow (microgametes ?). In the crow he found a hematozoön which was 100–130 μ long, and narrower than a red blood cell. It is more likely that this was a filaria rather than a trypanosome.

Similarly, Wedl's observations[93] leave it by no means certain that he saw trypanosomes. In the fresh blood of the cherry-finch (*Loxia coccothraustes*) he found filaria and oval bodies of about the size of red blood cells. These bodies were provided at one end with a crown of cilia, and were regarded by Danilewsky as trypanomonas.

In view of the indefiniteness of these early observations it is but proper to credit Danilewsky with the discovery of bird trypanosomes. His first paper[8] on this subject appeared in 1885, and four years later a very extended description was published in book form.[12]

Danilewsky examined more than 300 birds, but gave no details as to the frequency and number of the trypanosomes found by him. They were met with in the blood of owls, rollers, lannerets, etc., and the number was said to vary with the individuals and the season. In one case they were found in young rollers (*Coracias garula*) but three or four days old. They were also found in young featherless lannerets. He observed only one form of trypanosome which to him corresponded perfectly with the *Tr. fusiforme* (*piscium*) of fish. According to the size he divided this into *Tr. majus* and *Tr. minus*. The length of the latter, not counting the flagellum, varied from 18–22 μ; while that of the former was 45–60 μ. The young forms (9–10 μ), arising by segmentation, he designated as *trypanomonas*. Whereas in the heart-blood but one or two trypanosomes could be found, and then only with difficulty, in the red marrow of bones they were detected in large numbers. It would seem as if the red marrow was the principal place where these organisms are found.

Although Danilewsky designated the trypanosomes by several names, such as *Tr. fusiforme*, *Tr. majus*, *Tr. minus*, *Tr. sanguinis avium*, *Tr. fusiforme avium*, *Tr. costatum*, it must not be assumed that these meant to him distinct species. On the contrary he held to a unity among the trypanosomes just as he did for the cytozoa of birds. Nevertheless, it is evident that he met with at least two distinct forms in respect to size. Our own results tend to confirm his view that these two forms represent stages in the development of one and the same species.

Grassi and Feletti[29] mention the presence of trypanosomes in bats, but accord no notice to those of birds, other than to express their conviction that they have nothing to do with the malarial parasites.

According to Sjöbring[88] trypanosome infection of birds is widespread about Säftstaholm in Södermannland, Sweden. He was able to find the organism in nearly all passerine birds examined by him with the exception of Corvus and Pica. The infection was apparently local, for elsewhere he did not meet with it. Beyond this mere statement he gave no detail, and merely added that the forms observed corresponded with those of Danilewsky.

Ziemann,[94] in 1898, in 190 birds examined found trypanosomes but once and that in a chaffinch (*Fringilla cœlebs*) from Heligoland. Beyond the mere mention of this fact nothing further is said. The title of a more recent paper by Ziemann,[95] which indicates the presence of a trypanosome in an owl captured in Cameroon, is misleading. As a matter of fact, he did not find a real trypanosome but, acting upon a personal communication from Schaudinn, he used this term to designate a "leucocytozoön."

According to Laveran,[55] trypanosomes are very rare in birds indigenous to France, inasmuch as he examined a large number with negative results. In an owl (*Syrnium aluco*), purchased in Paris, he found trypanosomes, but they were very scarce. This bird had a quadruple infection: filaria, *H. Danilewskyi*, *H. Ziemanni*, and Trypanosoma. The latter, whip included, measured 33–45 μ. Following Danilewsky, Laveran designated this species as *Tr. avium* and carefully described its characteristics.

Dutton and Todd,[25] on their expedition to Senegambia, were able to find a larger number of trypanosomatic infections than any of their predecessors. Of 40 birds examined eight were found to have trypanosomes in their blood.

At Bathurst, 25 birds (mostly *Estrelda* and *Crithagra*) were examined and of these only one was infected. The scarcity of the parasites in the blood may be seen from the fact that they found but from two to four trypanosomes in a cover-glass preparation. This particular species they designated as *Tr. Johnstoni*. It had a very active spirillum-like motion, but possessed no free flagellum, and the undulating membrane was scarcely recognizable. The body was long, straight, and pointed at both ends. It measured 36–38 μ in length, and 1.4–1.6 μ in width. Two larks were inoculated with the blood from this bird, but with negative result.

At St. Louis, Senegal, they examined 15 birds (*Estrelda* and *Crithagra*) and found seven of these to be infected. The species in this case was left undetermined. They were not present in large numbers, were stumpy, sluggish, and of great width. The body measured 21.6 by 8 μ; the length of the flagellum was 10–12 μ. Two pigeons and two larks were inoculated with negative results.

Hanna,[35] while in India in 1900, examined the blood of domestic pigeons which were infected with *H. Danilewskyi*, and found trypanosomes. The percentage of infected birds was not large, and the parasites were comparatively few in number. The health of the birds was not affected. The trypanosome described and figured by Hanna is 45–60 μ in length, and 6–8 μ in width. A feature which distinguishes this trypanosome from that of Laveran is the transverse position of the nucleus and the nearness to this of the blepharoplast. In this respect it agrees, it may be added parenthetically, with one of the forms obtained by ourselves.

In this same paper Hanna gives a description of some trypanosomes present in preparations made from the blood of crows by Ross, while in India, in 1898. Apparently the two forms represent different species as can be seen from the following measurements:

	Pigeon	Crow
Length of organism	45–60 μ	40–56 μ
Breadth opposite to nucleus	6–8 μ	3–4.8 μ
Length from centrosome to posterior end	19–22 μ	8–9.5 μ

In this connection it may be stated that Laveran and Mesnil[57] cite a personal communication from Donovan, who found trypanosomes at Madras in the blood of an owl (*Athene brama*).

In 1903 Edm. and Et. Sergent[86] examined 307 birds in Algeria. It is worthy of note that all but 18 harbored one or more parasites. Thus, 37 had *Hæmamœba relicta*, 155 had *H. Danilewskyi*, two had *H. Ziemanni*, 42 had filaria, and six had trypanosomes. The latter organisms were extremely rare, and were found only in the fresh blood, not in the stained preparations. We have found this to be usually the case in our own examinations. The trypanosomes were present in one out of 46 goldfinches (*Fringilla carduelis*), in two out of five black-caps (*Sylvia atricapilla*), and in three out of 10 swallows (*Hirundo*) examined. No detailed descriptions or measurements are given.

Early in 1904 Schaudinn[85] reported his studies upon the owl (*Athene noctua*), in which he found proteosoma, halteridium, and *H. Ziemanni*. The last two organisms, for reasons already given, he designated as *Trypanosoma*

noctuæ and *Spirochæta Ziemanni* respectively. By inference, rather than from actual statement, it is clear that trypanosomes were present in the naturally infected birds.

This belief is substantiated by the recent work (July, 1904) of the Sergents[87] upon owls in Algeria. They were able this time to find trypanosomes, in very small numbers, in stained preparations, and confirmed Schaudinn's observations with reference to the presence of trypanosomes in the digestive canal of mosquitoes which sucked the blood of this owl.

The presence of trypanosomes in the stomach of mosquitoes was observed prior to the work of Schaudinn and of the Sergents. Thus, Chatterjee[6] is quoted by Rogers[76] as having found trypanosomes in the anopheles near Calcutta. Not having access to the original paper we are unable to state whether the origin of this infection was established. Another illustration on this point is that given by Durham[24] in his report of the yellow fever expedition to Parà. Some specimens of *Stegomyia fasciata* were placed in a cage with a bat, and when examined later showed trypanosomes. It is probable that these came from the blood of the bat which, however, had not been previously examined. The presence of trypanosomes in bats has been noted by Grassi,[29] Dionisi,[21] Testi,[89] and by Donovan (cited by Laveran and Mesnil[57]).

Finally, to this list of observations bearing upon the presence of trypanosomes in the blood of birds must be added the recent discovery (May, 1904) by Levaditi of a trypanosome in the blood of a Java sparrow (padda or rice-bird, *Padda oryzivora*) purchased in Paris. The trypanosome is evidently rare in these birds, for Laveran[52] examined, at one time, a large number without finding any other parasite than *H. Danilewskyi*. This trypanosome has been designated by Laveran and Mesnil[57] as *Tr. paddæ*, who have also given a very full description of this new organism. Thiroux[90] working under their direction has studied this trypanosome and the results have been recently published. From the illustrations and description given by him we are inclined to consider this as probably identical with the large form of Danilewsky, the trypanosome of Hanna, and the large type found by us. The question of identity, however, can not be settled owing to the absence of a comparison of the cultures of these organisms.

METHODS EMPLOYED.

Before discussing the results obtained in this investigation it may be well to give in detail the methods which we have employed. These, in general, cover the direct detection of trypanosomes in the blood, their cultivation, and the inoculation of birds with such pure cultures.

The necessary drop of blood for the direct examination was usually obtained from the marginal vein on the inner side of the wing. For this purpose the feathers were first removed, and the skin was then washed with a little water. By means of a pair of sharp-pointed scissors the small vein was cut, and a drop or two of blood was thus obtained. The resulting injury is so slight that the smallest bird, such as a sparrow or canary, can be examined every few days during a long period of time. The selection of a small vessel gives just enough blood for the examination, and obviates the excessive bleeding which is likely to occur when a larger blood-vessel is cut.

In the case of the dead bird, the blood was taken directly from the heart, either by means of a fine Pasteur pipette, or with the aid of a platinum wire, or by direct contact of the cut surface with the cover-glass.

When examining the fresh blood under the cover-glass care was taken to apply just enough pressure so as to obtain a single layer of blood corpuscles. When this is properly done it becomes a very easy matter to detect intra-cellular parasites, even when these are present in very small numbers. This is especially true of the parasites of the red blood cells which are easily recognizable by their hyaline bodies, the "pseudo-vacuolae" of Danilewsky, and by the presence of the blood pigment or melanin. The so-called "leucocytozoa" are less easily recognized because of their resemblance to white blood cells, and the almost total absence of pigment granules. This is particularly true when their number is very small. The formation of microgametes, especially during the morning hours, is very prompt, and may attract attention where otherwise the parasite would be unnoticed.

The detection of trypanosomes in the fresh blood is by no means an easy matter, owing to their extreme scarcity. And yet this method is, as a rule, more delicate than the examination of stained preparations. When, for instance, there is but a single trypanosome in the blood under the cover-glass, it is more likely to attract attention by its movements and the agitation of the blood corpuscles than is the stained preparation in which the organism is, likely as not, concealed by a mass of cells. We have repeatedly been unable to find trypanosomes in stained specimens of blood the direct examination of which had readily revealed their presence.

This statement holds true also for the very small free hemogregarines or vermiculi. Notwithstanding their small size, their peculiar motion, and that of the blood cells with which they come into contact, as well as their characteristic refractile appearance, assists materially in their detection.

The fresh blood preparation should be examined first for the presence of filaria with a low power, such as a No. 3 Leitz objective. These forms, owing to their large size, can be readily detected thus, even when but one or two chance to be present in the specimen. The No. 7 objective is sufficient to show the other organisms, and is especially useful for rapid orientation. Of course, the oil-immersion must be used to establish the nature of doubtful parasites, and to bring out further details.

The method of staining which we used in the early part of our work was essentially that of Romanowsky as modified by Nocht. The solutions employed were:

1. An aqueous one per cent solution of eosin (Höchst).

2. An aqueous one per cent solution of medicinal pure methylene blue (Höchst).

The latter must be "ripened" before use. This is accomplished by adding one-half per cent of crystallized sodium carbonate, and allowing the solution to stand for several days in a paraffin bath at about 60°. A better and more rapid procedure is to place the flask containing the solution in a boiling water-bath for about half an hour, and during this time to pass through the liquid a stream of steam. The latter is generated in a flask from which it is carried into the solution through a drawn-out piece of glass

tubing. To this product one-half volume of one per cent methylene blue solution is then added.

The actual staining mixture is made by adding to about 10 c.c. of distilled water in an Esmarch or Petri dish three or four drops of the eosin solution. By slight stirring the eosin is distributed through the liquid after which about from two-thirds to one c.c. of the "ripened" or polychrome methylene blue solution is then added, and the whole stirred.

The blood smears, either on cover-glasses or on glass slides, are first fixed by immersion for about 10 minutes in a mixture of equal parts of absolute alcohol and ether. The cover-glasses are then floated on the staining mixture with the specimen side down. When slides are used these are also turned with the specimen side down, but with one end resting on the edge of the tilted Esmarch or Petri dish.

The specimens are allowed to remain in contact with the dye for about 15–20 minutes after which they are rinsed in tap-water and dipped for a few moments in an eosin solution of about one-half of one per cent strength. This serves to remove the excess of methylene blue, and imparts the desired contrast tint of eosin. The preparation is then rinsed and examined in wa'er, after which, if satisfactory, it may be floated off, dried, and mounted in Canada balsam. When properly prepared the specimens will retain their color for several years.

The formation of a dirty deposit on the specimen can be avoided by the use of absolutely clean cover-glasses and by the use of a fresh eosin solution. The latter is prone to deterioration, and hence should be freshly prepared, every week or two. The preparation which is properly stained will show, in the case of bird's blood, the nuclei of the erythrocytes stained a deep red, while the remainder of the cell has a slight eosin tinge. The nuclei of the large leucocytes are stained pink-red while the plasma is blue. The blood plates which are scattered through the specimen appear as irregular discs of a bright red color. The nuclei of the parasites are stained a more or less deep red, while the plasma takes on a blue of variable intensity. It is least stained in the case of proteosoma, slightly more with halteridium, and most strongly with the larger "leucocytozoa." In the case of the latter the plasma of the male cell is stained a pale blue, whereas that of the female cell is usually stained a very deep blue.

The bird trypanosomes do not apparently stain as readily as do those of the rat and of Nagana, for it is the exception to find specimens with well-stained nuclei and flagella. More often the space corresponding to the nucleus is colorless or nearly so. The flagella are usually very indistinct, but nevertheless their presence can often be demonstrated. When the stained preparation fails to show free flagella it must not be assumed that these are absent, for an examination of the fresh living parasite, as found in the blood and especially as present in the culture, will show their presence. At no time have we found trypanosomes with the terminal, free flagella absent.

The staining of cultures is even more difficult than of the blood preparations. Not only is it difficult to stain the nuclei and flagella, but the precipitation of dye upon the cover-glass is such as to make the specimen worthless. It is only recently that with the aid of Mr. Torrey we have been able to modify

the method of staining so as to obtain satisfactory preparations of this kind. The slides thus prepared have made it possible to secure the excellent photographic reproductions of the cultural trypanosomes shown in the accompanying plates.

The cultivation method, as will be shown later, is far superior to the direct examination, inasmuch as it enables the isolation of trypanosomes when apparently none can be detected by the microscope. The method employed is essentially the same as that which we have used in our work on *Tr. Lewisi*,[64] *Tr. Brucei*,[65 66] and *Tr. Evansi*.[70] In one respect, however, it has received an important modification. It will be remembered that in the work on *Tr. Brucei* only eight per cent of the infected animals gave cultures of this organism. In endeavoring to ascertain the reason for the failure in this large percentage of cases we found that the concentration of the meat extract, as used for the preparation of the agar, was a most important factor. The result of this inquiry has been published by one of us (Dr. MacNeal[60]). The main fact established is that an excess of meat extract inhibits, possibly by overstimulation, the development of the initial culture, whereas a smaller amount favors the growth. This is particularly true of the initial or first generation, but after this is once obtained the organism readily adapts itself to the ordinary medium.

Consequently, we have used in this work an agar prepared according to the following formula:

Extractives of 125 g. of rabbit or beef meat in 1,000 c.c. of distilled water; two per cent Witte's pepton; 0.5 per cent salt; two per cent agar; and 10 c.c. normal sodium carbonate solution.

The agar thus prepared is tubed and sterilized in an autoclave at 110° for 30 minutes. When cooled to about 50°, two volumes of defibrinated rabbit's blood are added, and the mixture is then allowed to solidify in an inclined position. When firmly set it is placed upright for a few minutes until a few drops of water of condensation appear. This liquid is then inoculated with a drop of blood taken from the heart of the bird by means of a drawn-out tube pipette. In some instances, as when large birds are used, the blood may be drawn from the median vein by means of a sterile syringe. The cotton plug of the tube is then cut off short, moistened with mercuric chloride, and the tube is covered with a rubber cap, after which, it is placed at 25° for about a week.

Under these conditions the bird trypanosomes grow readily and even luxuriantly. As already stated the cultures often succeed when there is no microscopical evidence of the presence of the flagellates. This fact in itself shows how easily they adapt themselves to the new medium. It may be safely said that their cultivation is as easy as that of *Tr. Lewisi*. As a rule it is possible to recognize the presence of growing trypanosomes in the tubes on the third day. On the sixth to the seventh day they are usually extremely abundant and very actively motile. The picture presented by such an active vigorous culture is interesting in the extreme, and varies considerably with the species of trypanosome under observation.

It will be seen that the cultivation method not only serves the purpose of demonstrating the presence of trypanosomes, but also furnishes in many cases

a positive means of differentiating species. This in itself makes it an invaluable addition to the purely morphological method of studying these protozoa. We have heretofore shown that the cultural characteristics of the Nagana, Surra, and rat trypanosomes are very different, and permit of their ready identification. It may be added that the bird trypanosomes, in culture, differ markedly from each of these three organisms as well as among themselves.

When cultivation is attempted it is always advisable to inoculate three or four tubes of the medium for the reason that it may happen that but one out of a set of such tubes may develop. This is readily understood when it is borne in mind that the number of trypanosomes may be very small, scarcely more than one in a drop.

As a routine we have found the following procedure useful: The blood is taken from the heart by means of a sterile pipette and transferred to four tubes of blood agar (one to eight meat extract), which are then capped and set aside at 25°. The remaining blood is used to make cover-glass or slide smears which when air-dried are fixed in a mixture of equal parts of alcohol and ether, after which they are stained by the Romanowsky method. After spreading the films a slide of the fresh blood is examined for hematozoa, first with the No. 3 Leitz objective, then with a No. 7, and finally, if necessary, with the oil-immersion lens. As a rule, this examination was made without the use of a movable stage. However, when examining stained and mounted preparations, it is advisable to make use of one, and for this purpose the new Zeiss model is to be recommended. This, in addition to being detachable, enables one to locate a given field, even when the slide is transferred to another microscope. Although not intended to be used with the microphotographic stand, we have so used it to great advantage.

The injection of the trypanosome cultures can be made either subcutaneously or into the breast muscle. The intraperitoneal injection we found to be rather dangerous, and for that reason we abandoned it in favor of intrapleural injections. These can be made with the greatest of ease and with the least possible injury to the bird. For this purpose the needle of the syringe is inserted obliquely through the furcular angle into the right pleural cavity. Relatively large doses, even one-half c.c., can thus be introduced into a small bird, such as a sparrow or canary. The feathers over the wish bone should be removed and the skin washed previous to making the injection.

UNSUCCESSFUL CULTIVATION EXPERIMENTS.

The immediate object of this investigation was to establish, if possible, the correctness of Schaudinn's views as to the trypanosome stages of the intracellular parasites. For this reason cultures were made, as a rule, from such birds as contained hemocytozoa. In some instances the cultures were made from birds which contained no recognizable parasites. In the case of the infected birds special care was taken to use those which were very rich in fully developed hemocytozoa, that is to say those which

gave rise to an abundance of microgametes. Nevertheless, such attempts were often fruitless, as will be seen from the synopsis which follows.

It may be stated in advance that of the tabulated 26 negative attempts at obtaining cultures (including those of three canaries), 19 were made with birds very rich in one or more intracellular parasites, such as *proteosoma*, *halteridium*, *H. Rouxii* with free hemogregarines, and *H. MacCallumi*. The remaining six birds had received previous injections of cultures of trypanosomes or other parasites. The failure in such indicates that, in the bird used, the trypanosomes, in the interval which elapsed, had disappeared from the blood. An instance of this kind was afforded by robin No. 270, in the blood of which trypanosomes were found 19 days before the culture was attempted. Only one tube was inoculated, and it failed to give a growth, showing that either the number of parasites had greatly decreased or had entirely disappeared in the interval.

Cultures were also attempted in the case of 10 robins which had a more or less intense infection with one or more of the following: *Pl. Vaughani*, *H. majoris*, *halteridium*, and *filaria*. Owing, however, to contamination with bacteria failure resulted. It is quite probable, judging by the large number of successful cultures obtained from robins, that some of these would have shown trypanosomes.

1. Sparrow, No. 53.—Received an injection of blood and internal organs of robin No. 51 which had numerous *halteridia*, a few *H. majoris*, and a few trypanosomes; died next day. Four tubes were inoculated; result, negative.

2. Sparrow, No. 76.—Received an injection of suspension of blood and organs of wren, No. 86, rich in trypanosomes. Two days later a few *proteosoma* appeared, these increased greatly, and on the fifteenth day, when very rich, it was etherized and four tubes inoculated. Result, negative.

3. Sparrow, No. 75.—Received same injection as preceding; repeated examinations negative. Twenty days later received injection of blood of robin No. 271 rich in *halteridium*, *H. majoris*, *Pl. Vaughani*. No infection; died 10 days after second injection. One tube inoculated, negative.

4. Sparrow, No. 108.—Received injection of blood of hemogregarine sparrow, No. 111. Four days later *proteosoma* were rich. Was etherized on the fifth day and four tubes inoculated; result, negative.

5. Sparrow, No. 113.—This sparrow was rich in *H. Rouxii* and in the long, free hemogregarines. Was etherized and two tubes inoculated; result, negative.

6. Sparrow, No. 123.—Extremely rich in *proteosoma*. Was etherized and two cultures made; result, negative.

7. Sparrow, No. 129.—Was like the preceding; two tubes inoculated with the heart-blood were negative.

8. Sparrow, No. 140.—Was inoculated with blood of *proteosoma* sparrow. Eleven days later, when extremely rich in segmenting forms, was etherized and four tubes inoculated. Result, negative.

9. Sparrow, No. 149.—Sparrow rich in *halteridium*. Was etherized and two tubes inoculated; result, negative.

10. Sparrow, No. 151.—Received an injection of blood of above *halteridium* sparrow. Eleven days later showed few *halteridia* and many *proteosoma*. Was etherized and two tubes inoculated. Result, negative.

11. Sparrow, No. 180.—Was inoculated with blood of *halteridium* sparrow, No. 149. No infection; died nine days later. One tube inoculated with negative result.

12. Sparrow, No. 181.—Was inoculated the same as preceding, and died also on the ninth day. No infection. One tube was inoculated with negative result.

13. Sparrow, No. 216.—Was extremely rich in all stages of *proteosoma*. Was chloroformed, and four tubes were inoculated. Result, negative.

14. Sparrow, No. 233.—Rich in *proteosoma* with few *halteridia;* was chloroformed and two tubes inoculated. Result, negative.

15. Sparrow, No. 234.—Was rich in *proteosoma* and *halteridia*. Immediately after death four tubes were inoculated with negative result.

16. Sparrow, No. 297.—Was very rich in *proteosoma*. Immediately after death two tubes were inoculated, but with negative result.

17. Sparrow, No. 307.—Was very rich in *halteridia* and in hemogregarines. Was chloroformed and one tube inoculated with negative result.

18. Sparrow, No. 313. Very rich in *proteosoma;* had some free gregarines: was chloroformed and two tubes inoculated. Result, negative.

19. Sparrow, No. 319.—Rich in all forms of *proteosoma* and in *halteridia;* was chloroformed and two tubes inoculated with negative result.

20. Sparrow, No. 338.—Was injected with a citrated suspension of cultures of strains A, B, C, D, E. Seven days later segmenting forms of *proteosoma* were found (latent infection). Died on the fourteenth day. One tube was inoculated with negative result.

21. Sparrow, No. 339.—Injected the same as the preceding; also died on the eleventh day. No infection. One tube was inoculated with negative result.

22. Chipping sparrow, No. 263.—*Halteridia* very abundant. Immediately after death three cultures were made. Result, negative.

23. Mourning dove, No. 85. *H. MacCallumi* numerous. Blood drawn from vein with syringe. Four tubes were inoculated. Result, negative.

24. Canary, No. 1.—Received an injection of trypanosome cultures, strains J. K. L. Four days later was killed by accident. Two tubes were inoculated at once, but with negative result. No infection.

25. Canary, No. 2.—Received the same injection of trypanosome cultures as sparrow No. 20 above. No infection. Was killed sixteen days later and one tube inoculated. Result, negative.

26. Canary, No. 8.— Was injected with *proteosoma* blood of sparrow. Died ten days later with *proteosoma* very abundant. Three tubes were inoculated with negative result.

The failure to obtain cultures of trypanosomes from birds richly infected with hemocytozoa may be taken to show that these two classes of parasites are entirely distinct. This conclusion will be strengthened by other observations.

Recognized cases of trypanosomatic infection.

The following table is a summary of the findings in birds having trypanosomes. These it will be seen comprise 15 species and 38 cases. The second column indicates the result of the microscopical examination of the blood in the fresh condition. A negative result is shown by the sign —. The third column gives the findings in stained preparations of the blood. An ordinary examination of such stains was very often negative and for that reason the slides were examined, field by field, with the aid of a movable stage. In some instances the blood was spread on the slide and such smears were covered with a glass slip 21 × 42 mm. Usually, however, cover-glasses were employed which were about 21 mm. wide. In several cases (as in S and P), three or four slide smears were examined before the one trypanosome was found. Obviously, the detection of trypanosomes in the blood when present in such small numbers is largely a matter of chance.

The fourth column shows the results obtained by the cultivation method. As will be seen at a glance this procedure offers the surest means of detecting the trypanosomes. In only one instance did the method apparently fail, and that was in the case of robin, No. 270, in which the parasite was seen 19 days before the culture was attempted. The dotted sign (.....) in this column means that cultivation was not tried. The letters designate the strains isolated, and the numbers the generations or sub-cultures through which each was carried.

The fifth column gives the name of the species found, based upon the characteristics given later on. A number of these are open to question since the cultural or other characteristics are somewhat different from the type species.

TABLE I.

SHOWING RESULTS OBTAINED WITH BIRDS HAVING TRYPANOSOMES.

		TRYPANOSOMES DETECTED IN			SPECIES	OTHER PARASITES PRESENT
		Direct	Stain	Cult're		
1. Blackbird, red-winged (*Agelaius phœniceus*)	No. 401	−	+ 1 small 2 large	.....	*Tr. avium*	Filaria only.
2. " rusty (*Scolephagus carolinus*)	No. 420	−	−	A 10	Tr. sp.? Type 4	None.
3. Bluebird (*Sialia sialis*)	No. 426	−	−	B 8	*Tr. avium*	Filaria only.
4. Blue jay (*Cyanocitta cristata*)	No. 244	+	+ 8 small 18 large	K 10	" "	*Hæmoproteus majoris.*
5. " " "	No. 273	−	−	M 2	Tr. sp.? Type 4	None.
6. " " "	No. 278	−	+ 18 small 4 large	R 12	*Tr. avium*	*H. Danilewskyi*, very rich, very few *H. majoris.*
7. " " "	No. 408	−	−	Z 9	" " ? Type 1*a*	Filaria only.
8. " " " "	No. 429	−	−	C 9	Tr. sp.? Type 4	*H. Rouxi?* Gregarines?
9. Dove, mourning (*Zenaidura macroura*)	No. 5	+	+ 5 small	A 7	*Tr. avium*? Type 1*a*	*H. Sacharovi*, very abundant.
10. " " " "	No. 6	+	+ 1 "	B 22	" " ? Type 1*a*	" " " few.
11. Gold-finch, American (*Spinus tristis*)	No. 353	−	+ 1 "	S 10	*Tr. Laverani*	" few; *H. Danilewskyi*, rich.
12. " " " " "	No. 354	−	−	X 9	Tr. sp.? Type 4*a*	None.
13. Flicker (*Colaptus auratus*)	No. 388	−	−	U 10	*Tr. avium*? Type 1*a*	None.
14. Hawk, red shouldered (*Buteo lineatus*)	No. 350	−	+ 1 large	P 11	*Tr. Mesnili*	*H. Ziemanni*, *H. Danilewskyi.*
15. Oriole, Baltimore (*Icterus galbula*)	No. 41	+	+ 92 small	C 5	*Tr. avium*	None. *H. Rouxi*?
16. " " " "	No. 272	+	+ 8 "	.....	" "	None.
17. Robin (*Merula migratoria*)	No. 46	−	−	AB 4	" "	*H. majoris*, very few.
18. " " "	No. 50	−	+ 2 small	G 4	" "	*Pl. Vaughani;* few *H. Danilewskyi.*
19. " " "	No. 51	+	+ 9 "	D 5	" "	*H. majoris*, rich in *H. Dan'l'skyi.*
20. " " "	No. 53	+	+ 1 "	E 4	" "	" " " " "
21. " " "	No. 54	−	+ 9 "	F 1	" "	" " " " "
22. " " "	No. 184	−	−	I 2	" "	" " few.
23. " " "	No. 214	+ 3 large	−	L 2	" "	" " "
24. " " "	No. 242	+ 2 large	−	J 2	" "	" " "
25. " " "	No. 257	−	−	Q 12	" "	" " "
26. " " "	No. 270	+	+ 1 small 1 large	− ?	" "	" " " Culture attempted 19 days after tr. were seen.
27. " " "	No. 284	−	−	N 2	" "	*H. Danilewskyi*, few.
28. " " "	No. 345	−	−	O 1	" "	None.
29. " " "	No. 376	−	−	V 12	" "	*H. Danilewskyi*, very few; filaria.
30. " " "	No. 393	−	−	W 9	" "	" " *Pl. Vaughani.*
31. Sparrow, English (*Passer domesticus*)	No. 109	+ 1 large	−	.....	" "	None.
32. " " " "	No. 279	+ 2 large	−	.....	" "	None.
33. " " " "	No. 286	+ 1 large	−	.....	" "	None.
34. Song sparrow (*Melospiza fasciata*)	No. 142	−	+ 2	H 4	" "	*Pl. relictum*, very rich.
35. " " " "	No. 288	+	+ 28 small 1 large	.....	" "	*H. Danilewskyi*, rich.
36. Brown thrasher (*Harporhynchus rufus*)	No. 386	+	−	T 8	Tr. sp.? Type 3*a*	Filaria.
37. Woodpecker, hairy (*Dryobates villosus*)	No. 391	−	+	.....	Tr. sp.?	None.
38. House wren (*Troglodytes aedon*)	No. 86	+ 10	−	.....	Tr. sp.?	None.
Microscopical detection failed entirely in 14; these recognized by culture		16	17	29		15 without intracellular parasites

The last column gives a list of the other parasites present in each bird. Several of the names are of new species which will be described in the next paper.

The above table embraces the results obtained from an examination of 431 birds, representing 40 species. Of this number 38 (16 species) or 8.8 per cent were found to contain trypanosomes. This figure must not be taken to indicate the actual number of birds infected with these organisms for the reason that cultures were not attempted in all cases.

The actual number of cultivation experiments (free from contamination) made with wild birds, as seen from this table and the preceding summary, is only 53. Twenty-four of these were negative and 29 were positive. Of the cultural attempts, then, 55 per cent were successful. In eight other birds trypanosomes were found with the microscope, and, if at the time an attempt at cultivation had been made, judging from the success under like conditions, the cultures would probably have developed, in which case 37 out of 61 would have been positive, or about 60 per cent.

This statement may be somewhat misleading, for the reason that of the 61 birds in question, 18 were known, from the microscopical examination made at the time, to have trypanosomes. If, therefore, these are deducted, it will be seen that in the 43 birds in which the original microscopical examination failed to show trypanosomes the cultural method revealed their presence 19 times, or in about 44 per cent.

It is evident from these considerations that the percentage of birds infected with trypanosomes is much higher than would be indicated by the limited findings given in the table. It is probable that the careful application of the cultural method to a large series of birds will show that fully one-third, if not more, harbor these parasites.

It is certainly remarkable that the microscope, even after most careful re-examination, should show trypanosomes in only 24 out of 431 birds, whereas the cultural method applied in only 53 cases, excluding attempts in which contamination occurred, should give 29 positive results.

The relation of the intracellular parasites to the trypanosomes

is of especial interest. In connection with the summary of the unsuccessful cultivation trials it was pointed out that, although cytozoa were very abundant in 19 of those cases, yet no culture was obtained. By contrast the above table shows that trypanosomes may be present, at times in very appreciable numbers, unaccompanied by intracellular parasites. Cytozoa were absent in 15 and present in 23 of the birds known to have trypanosomes.

The existence of a latent infection with cytozoa in these 15 cases is possible, but even with that assumption it is difficult to reconcile this finding with the supposed relation of trypanosomes to this class of organisms. Moreover, it can be shown that birds may harbor trypanosomes for weeks and months without showing any infection with intracellular parasites.

In one instance, in particular, a canary developed trypanosomes in its blood four days after an injection of a culture of strain D. The trypanosomes were found at times, though not always, during the following 11 weeks, when the bird died, but at no time was there any indication of the presence of a cytozoön.

Thiroux,[90] the only one previous to ourselves who has succeeded in infecting birds with trypanosomes, found that the period of incubation ranged from a few (12) hours to 18 days. This variation was due, in part, to the natural resistance of the bird (padda), and, in part, to the method of infection. The inoculations were subcutaneous, intramuscular, intravenous, or intraperitoneal; the latter being the surest method. The infection was of variable intensity, in some cases the parasites being scarce, while in others they were very numerous. Indeed, one of the padda birds died apparently from the severe infection which developed. In the case of moderate infection, usually following a short period of incubation, the trypanosomes increased in numbers during a period of 9 to 15 days after which they decreased and finally remained stationary, in some instances during a period of 40 days. In addition to the padda, five other species of birds, including the green and common canary, were successfully infected.

The significant feature of Thiroux's experiments is that, notwithstanding this rich experience in the artificial infection of birds with trypanosomes, no mention is made of the appearance of

intracellular parasites which it is reasonable to expect would appear if the latter were but stages in the life-history of the former. The fact that no mention is made of the presence of cytozoa leads us to believe that they were not found. With this assumption, his results are in accord with ours on the canary mentioned above. They agree furthermore with the observed fact noted in the table that trypanosomatic infection of wild birds may exist without association with intracellular parasites.

Furthermore the study of the trypanosomes found in the blood, and also of those obtained by cultivation, shows that there are several distinct species which exhibit no constant association with a given cytozoön. Thus, the most common species, described further on as *Tr. avium*, was associated with *H. Sacharovi*, *H. majoris*, *H. Danilewskyi*, *H. Rouxi*, *Pl. Vaughani*, or with *filaria* in addition to its very frequent single occurrence. Under these circumstances it would indeed be difficult to establish a relationship with any of the intracellular organisms mentioned.

Morphology of Trypanosomes in Blood.

Observations made upon the trypanosomes found in the fresh blood or in stained preparations are insufficient for the purpose of identification. As in the case of bacteria, the cultural characteristics and the animal experiment must, so far as possible, be utilized in order to differentiate allied organisms. In the study of the trypanosomes of mammals it has been, after all, the animal experiment which has served the purpose of identification, since the morphological variations are at times so slight as to be almost negligible. In three of these infections the cultural method has supplied an additional means of recognition. Without doubt, the number of species of trypanosomes met with in mammals and man is very large. Moreover, with perfected methods it will be found that a given species of mammal may be subject to natural infection with several kinds of trypanosomes, some of which may be very pathogenic, while others are not. In other words infections of this type will be found to parallel those of other protozoa and even of bacteria. It will be sufficient to take a single illustration—that of malaria. Malarial infection, as met with in man,

monkeys, bats, and birds, is due to different species of parasites. And, in the same species of animal, as man or bird, several species of these parasites may be found. That it is possible for one species of bird to harbor two kinds of trypanosomes will be shown farther on.

As indicated above, a thorough study of a given trypanosome embraces observations upon the living and stained organism as found in the blood, an examination of its cultural characteristics, and a determination of the pathogenic action of the pure cultures upon the same species as the host, as well as upon other species. It has not been possible to meet all these requirements in every case of trypanosome infection in the present investigation for reasons which will be readily seen.

In the 24 cases recognized by means of the microscope, the trypanosomes were found in the living condition in the blood only 16 times. The stained preparations showed them to be present in 17 cases. In 14 of the birds the examination of the fresh and stained blood was negative, the organisms being detected solely by the cultural method. It should perhaps be stated that the number of positive stains would have been much less than that given above were it not for the very valuable check afforded by the cultivation process. It happened several times that the ordinary examination of the stain was negative, but inasmuch as the culture eventually developed it led to a re-examination of the slides, field by field, and in a few instances the laborious search was rewarded by finding the trypanosomes in the stains. In the case of culture S (*Tr. Laverani*) only one trypanosome could be detected thus on three slides made from the original blood. Similarly, only one could be found on four slides of the blood which gave culture P (*Tr. Mesnili*).

The difficulty of detecting trypanosomes in stained preparations can best be shown in the case of a canary which was artificially infected by means of culture D. During a period of 11 weeks the parasites were repeatedly found in living condition in the blood, and, although stains were made at the same time, they were invariably negative. When the bird died trypanosomes were found as usual, but, although seven slide smears

were made and carefully examined on a movable stage, the examination consuming about 20 hours, the result was the same as before.

With but few exceptions, the trypanosomes which we found in the living state in the blood were one of two types, corresponding very closely to the *Tr. majus* and *Tr. minus* of Danilewsky, so much so that we are obliged to consider our common forms as identical with the ones described by him. Moreover, we are in accord with Danilewsky in regarding these two forms, notwithstanding their great difference in size, as belonging to one and the same species—*Tr. avium*. This statement, however, refers only to the common trypanosomes of birds since, as will be seen, there are several species.

In five of the birds, as can be seen from the table, the large and small forms were found together. At first sight, it would appear as if the two forms represented distinct species, but that such is not the case is readily seen from the cultural characteristics. In other words, the cultures made from the blood of birds having only the small form, or only the large form, or both forms at the same time, present exactly the same appearance. The position of the blepharoplast with reference to the nucleus clearly shows that the large form is to be considered as preparing for division. Furthermore, the presence of this type in very young birds indicates that the infection is of recent origin. In this respect it corresponds to the large form of *Tr. Lewisi* which appears in the blood on about the sixth to the ninth day of infection. As is well known, in the rat after the stage of active multiplication of *Tr. Lewisi* is passed, the large form disappears, and only the ordinary or typical form persists. That there is a close generic relation between *Tr. Lewisi* and *Tr. avium* is seen in the marked similarity of the cultures, both giving rise to actively motile free-swimming forms, and to characteristic multiplication rosettes, the individuals composing which have their flagella directed toward the center.

In view of these facts we are led to regard this large form as a multiplication, or possibly sexual type, of the smaller and more common form.

This large form usually appears as an S-shaped body having

a well-developed, undulating membrane, which terminates in a free flagellum 10–15 μ long. The body proper varies in length from 21–30 μ, but at times it may attain even 40 μ and more. The posterior end for about 12–15 μ is narrow and tapers to a point. It shows a peculiar stiffness, due without doubt to the fact that the blepharoplast is near the nucleus. The nucleus is readily visible in the living form as a large round body, or, more often, as an oval which is placed transversely across the body, filling out the entire width. The width of the cell opposite the nucleus is from 5–7 μ. As might be expected, owing to its large size, this form shows very little tendency to travel out of the field of the microscope, and for that reason can be readily kept under observation for hours. The contortions, however, are very active, and the organism is constantly changing from side to side, at times straightening out, or forming a coil. For that reason the measurements as given above, made on the living parasite, are only approximate.

The stained preparation shows the large form in the characteristic S-shape (Plates 2 and 3) or in the circular position (Plates 3 and 4). The centrosome, it will be seen, lies close to the nucleus and is in a large colorless space. The nucleus itself does not stain readily, and for that reason appears as a large light body in the middle of the cell. Beautiful striæ or myonema lines, six or eight in number, are easily made out on the part overlying the nucleus. These lines are continued the entire length of the cell. The long stiff or atrophied posterior end is very noticeable. The undulating membrane is visible as a delicate, fairly wide, and rather wavy border on one side of the organism. As will be seen from the table, the length of the body of the large form varies within wide limits, that is from 35–65 μ. The whip is probably 15–20 μ long.

The two forms described represent the common types as met with in the blood of birds, and are to be regarded as belonging to the same species. Two other trypanosomes, however, were found in the blood.

The first of these met with, in the blood of a hawk, is shown in Fig. 1, Plate 5. The large size (see culture P, Table 2) and peculiar shape mark it at once as distinct from the preceding.

Moreover, the cultural characteristics are totally different and stamp it as a new species. We have named it *Tr. Mesnili* in honor of Dr. Mesnil, of the Pasteur Institute. The full description of this form will be found later on.

The second trypanosome, referred to above, was found but once in the stains made from the blood of a goldfinch. It is designated as Culture S in the table, and from the measurements there given it will be seen to correspond closely to the ordinary small form of *Tr. avium*. Indeed, if the size was the only criterion, there would be little hesitation in regarding it as being of the same species. The presence of a large number of granules in the posterior half would hardly be sufficient evidence for separating it as a distinct species. And yet the cultural features of this organism are unlike those of *Tr. avium* or of *Tr. Mesnili*, and on that account it must be considered as a new species. We have designated this organism as *Tr. Laverani* in honor of Dr. Laveran.

The small form is the most common one met with in the blood of birds. The total length, whip included, is about 25–30 μ, or a trifle more than the length of two blood corpuscles. The body proper is about 20 μ long, though exceptionally it may vary from 14 to 25 μ. The width ranges from 3.5–5 μ. The nucleus, of nearly the same width as the cell, is at times visible, but not always. The posterior half of the body usually shows minute granules or globules, but at no time is there any indication of pigment granules. The body is usually in the form of a straight spindle, widest in the middle, tapering quite evenly in both directions. The anterior end is usually bent at right angles to the line of the body. There is an absence of the stiff posterior end as met with in the larger form. The undulating membrane is easily made out, though it is not as wide as in the other type. It starts very close to the posterior end, and as it passes forward, crosses the body, forming one or two wavy bends. It finally terminates in a free flagellum, 8–12 μ long. The motion is sluggish, and, as in the other form, there is but little tendency to travel out of the field of view.

The small form as seen from the photographs (Plates 1–4) is a spindle-shaped body which tapers to a sharp posterior end.

The large centrosome or blepharoplast at times appears to be at the very tip, while again it may be four or five μ distant. It is often surrounded by a colorless or achromic zone. The flagellum, in several of the preparations, is seen to start from the centrosome and form the outer border of the undulating membrane, eventually becoming the free whip, which is about 8–10 μ in length. The nucleus is not always distinct, but it can often be seen as a large body nearly as wide as the cell. In the preparations which have become crushed or flattened out (see Fig. 4, Plate 1, and Fig. 2, Plate 3) the nucleus and border of the undulating membrane can be seen very plainly. In a few instances a faint suggestion of striæ on the surface of the body can be made out.

The description and measurements of the two forms as given above, unless otherwise indicated, refer to the organisms as found in the living condition. Obviously, it is not possible on account of the constant movement to make very accurate measurements, whereas with the stained preparation this can be done very satisfactorily. The stain has the further advantage in that structural differences are readily brought out, if any exist.

The measurements given in the appended table include all the kinds of trypanosomes met with in the stained preparations. In the few instances where the number of organisms was large, only five or six were taken for this purpose.

The small forms are grouped together in order to show the close similarity in size. At first sight it would seem as if there were two types, one of which was about 20 μ in length with the centrosome close to the posterior end, usually not over 1 μ from the tip; while the other was about 25 μ in length with the centrosome about 5 μ from the end. In view of the fact, however, that the cultural features are so closely alike, if not identical, it has not seemed desirable at present to place too much stress on this slight difference, especially as a still greater variation is met with in the case of the large form which, as mentioned above, must be regarded as belonging to the same organism. It is quite probable the larger of the small forms, with its centrosome at a distance from the tip, constitutes a transition to the large form which has its centrosome close to the nucleus.

TABLE II.

GIVING MEASUREMENTS OF TRYPANOSOMES AS FOUND IN STAINS.

Trypanosomes Found in		Length of Free Flagellum	Length of Body	Distance from Centrosome to Posterior End	Distance from Centrosome to Anterior End	Distance from Centrosome to Center of Nucleus	Width of Body at Nucleus	Size of Nucleus	Length of Centrosome
Baltimore oriole, No. 41, culture C.	*a*..	8.0	21	1.0	20	10	5.0	3×5	0.7
	b..	....	20	0.5	19.5	10	5.0	4×3	0.7
	c..	....	20	1.0	19	12	5.0	5×4	0.7
	d..	....	21	0.5	20.5	10	4	4×3	0.7
Robin, No. 53, culture E.	*a*..	....	20	0.5	19.5	11	5.0	2.5×3	1.0
Robin, No. 270, No culture. Small form.	*a*..	8.0	20	0.5	19.5	12	5.0	4×4	1.0
Blue jay, No. 244, culture K.	*a*..	10	18	1.0	18	11.5	5.0	4×3	1.0
Small form.	*b*..	....	19	1.5	17.5	8.5	5.5	5×3	0.5*
	c..	....	16	1.5	14.5	9.5	5.2	5×3	0.7*
	d..	....	18	1.0	17.0	9.0	4.0	4×2	1.0
	e..	....	19	1.0	18.0	9.0	4.0	3×2.5	1.0
	f..	....	18	1.5	16.5	9.5	7.0	6×3	1.0*
Goldfinch, No. 353, culture S. A distinct species.	*a*..	....	20	1.0	18.0	8.	6.0	4×2	0.6
Song sparrow, No. 142, culture H.	*a*..	9.0	20.0	1.5	18.5	8.5	4.5	4×3	0.6
	b..	....	19.0	1.5	17.5	9.5	9.0	6×3	0.7*
Mourning dove, No. 5, culture A.	*a*..	6–8	22	1.0	21.0	10.0	5.0	4×4	1.0
	b..	....	23	1.0	22.0	10.5	5	4×5	0.7
	c..	....	22	1.0	21	9.5	4.0	3×4	0.7
	d..	8	22	1.0	21	9.0	4.5	3×4	0.5
	e..	....	24	1.0	23	10.0	4.5	4×4	0.7
Mourning dove, No. 6, culture B.	*a*..	8–10	24	1.0	23	10.0	5.0	4×4	0.7
Robin, No. 50, culture G.	*a*..	10	16	1.5	14.5	6.5	6.0	3×5	0.7*
	b..	9–10	23.5	5.0	18.5	6.5	6.0	3×5	0.5
Robin, No. 51, culture D.	*a*..	5.5+	23	4.0	19	12	5.0	4×5	0.7
	b..	9.0	25	5.0	20	10	5.0	4×5	0.7
	c..	....	25	5.0	20	10	5.0	4×5	1.0
	d..	....	25	5.0	20	10	5.0	4×5	1.0
	e..	....	24	5.0	19	9+	4.0	....	0.7
Blue jay, No. 278, culture R.	*a*..	9.0	24	6.0	18	7.0	5.0	4×4	0.5
Small form.	*b*..	13	25	5.0	20	7.0	5.0	4×5	0.5
	c..	8.0	24	5.0	19	8.0	5.5	4×5	0.5
	d..	....	24	5.0	19	7.5	5.0	3×4.5	0.5
	e..	7	24	5.5	18.5	8.5	5.0	3×5	0.5

*Indicates that the specimen was somewhat crushed or flattened. As a result it is shorter and wider than usual.

TABLE II—*Continued.*

Trypanosomes Found in		Length of Free Flagellum	Length of Body	Distance from Centrosome to Posterior End	Distance from Centrosome to Anterior End	Distance from Centrosome to Center of Nucleus	Width of Body at Nucleus	Size of Nucleus	Length of Centrosome
Robin, No. 54, culture F.	*a*..	....	20	4.0	16	8.0	5.5	2×5	1.0
	b..	8.0	21	4.0	17	7.5	5.0	3×4	1.0
	c..	....	20	3.5	16.5	8.0	5.5	3×5	0.7
	d..	....	20	4.0	16	7.5	4.0	2×3	1.0
	e..	....	22	4.0	18	8.5	5.0	3×5	1.0
Song sparrow, No. 288, No culture.	*a*..	8+	18	2.0	16	7.0	5.0	4×4	1.0
Small form	*b*..	....	17	4.0	13.0	6.0	4.0	3×3	0.7
	c..	....	19	3.0	15.0	10	3.5	3×3	0.5
	d..	....	17	2.0	15.0	8.0	4.0	3×3	1.0
	e..	....	20	3.0	17	8.0	4.0	3×3	0.7
	f..	....	21	1.5	19.5	10.0	5.0	4×4	1.0
Baltimore oriole, No. 272, No culture.	*a*..	10	20	4.0	16.0	7.0	5.0	2.5×2	0.5
	b..	8	20	5.0	15	8.0	6.0	2.5×3	0.5
	c..	6.0+	21	5.0	16	8.0	4.0	2×3	0.5
	d..	10	21	5.0	16	8.0	4.0	2×3	0.5
	e..	8	17	3.0	14	6.0	7.5	3×5	0.5*
	f..	7+	20	5.0	15	9.0	7.0	3×5	0.7*
	g..	7	22	6.0	16	8.0	6.0	3×5	0.7*
	h..	7	21	2.0	19	9.0	6.5	3×3	0.7
Blackbird, No. 401, No culture.	*a*..	....	35	15	20	6	4.0	3×3	1.0
	b..	....	35	15	20	6	5.0	5×5	0.7
Song sparrow, No. 288, No culture. Large form	*a*..	....	40	15.0	25	7.0	4.0	4×4	0.7
Blue jay, No. 278, culture R.	*a*..	....	53	19.0	34	7.0	5.0	4×5	0.5
Large form.	*b*..	20.0	50	17.5	32.5	6.5	6.5	5×5	0.5*
	c..	*Ca*.10	49	13.0	36	10	6.5	5×5	0.5
	d..	12	52	19.0	33.0	7.0	5.0	4×5	0.5
Blue jay, No. 244, culture K.	*a*..	....	50	20	30	7.0	5.0	4×5	1.0
Large form.	*b*..	10+	48	18	30	7.0	5.0	4×5	1.0
	c..	16.0	57	25	32	7.0	6.0	4×5	1.0
	d..	15 0	53	23	30	7.0	5.0	4×5	1.0
	e..	10–15	53	24	29	7.0	5.0	4×5	1.0
Robin, No. 270, No culture. Large form.	*a*..	....	65	20	45	7.5	5.0	5×5	1.0
Hawk, No. 350, culture P. A distinct species.	*a*..	....	50	7.0	43	11.0	8.0	2×3	1.0

*Indicates that the specimen was somewhat crushed or flattened. As a result it is shorter and wider than usual.

CULTURAL CHARACTERISTICS OF THE TRYPANOSOMES STUDIED.

Very little difficulty is experienced in cultivating the bird trypanosomes. As a rule they grow quite rapidly, so that at 25° their numbers are quite appreciable on the third day. They reach their maximum on about the seventh or eighth day after which they give rise to spherical involution forms which soon agglutinate or gather into large masses. These become coarsely granular, show highly refractive bodies, and eventually break down into a mass of granular débris. Living forms are rarely to be seen after about two weeks, although, exceptionally, we have met with them at the end of a month. When grown at room temperature, the culture naturally comes on more slowly and remains alive for a much longer period.

Of the 29 strains obtained from that number of birds, only two (strains S and T) showed a much slower growth. In these the trypanosomes were not appreciable until on about the sixth, and did not reach their maximum until about the tenth day.

In all cases the cultures when fully developed were enormously rich in the flagellates. At times there were observed on the surface of the blood agar, just above the fluid, circular colonies, two to three mm. in diameter, which were suspected to be due to bacterial contamination. An examination, however, of such colonies showed them to be a solid mass of actively wriggling trypanosomes.

Subcultures were obtained from all but two (strains F and O) which died out through oversight. In a number of instances these cultures have been kept up for over six months, during which time they have passed through more than 20 generations. Most of the strains have been kept up continuously for the past four months and continue to maintain their original characteristics. A number accidentally died out on account of delayed transplantation.

An examination of the 29 strains in the living condition shows at a glance that they comprise several distinct types, and it may be as well to speak of these for the present by that designation.

The division into types is based upon the characteristics shown by the rosettes and by the free-swimming forms. The main points in this grouping of the cultures can be summarized thus:

Type 1.—Rosettes and free forms common. The latter are very long and narrow, mere threads, without any noticeable enlargement of the body (*spirochetes*). Growth rapid. In these the blepharoplast is posterior to the nucleus. This is the most common type and corresponds to *Tr. avium.*

Sub-type 1a.—This is much like the preceding, but the spirochetes are shorter and are distinctly wider near the anterior end. They remain, however, long and narrow. Represented by strains A, B, U, and Z.

Type 2.—Large rosettes, the cells being of considerable size and having long central whips. The free forms are likewise large and wide, and show globules. Growth rapid. Represented by strain P—*Tr. Mesnili.*

Type 3.—Rosettes much less common and smaller. The free cells show relatively but little motion, have a blunt posterior end, and the contents are largely made up of big globules. Growth very slow. The cells show a posterior terminal rod. Represented by strain S—*Tr. Laverani.*

Sub-type 3a.—Growth equally slow as preceding. The free cells have a tapering posterior end. Represented by strain T.

Type 4.—Rosettes are very scarce, the free forms predominating. The anterior end of body tapers out gradually along the whip; the cell tapers also posteriorly, contains very small granules, and is actively motile. Rapid growth. Represented by strains M, A′, and C′.

Sub-type 4a.—This is represented by strain X. The rosettes are even more scarce. The growth is rapid and rich. The blepharoplast is very large.

Type 1.—This will be described as *Tr. avium* since it represents the most common form met with in birds. It was present in 18 out of the 29 cultures. In seven birds from which cultures, however, were not obtained the trypanosomes presented the characteristics of *Tr. avium.* It appears therefore that this species was present in 25 out of 38 birds. The particular birds in which this species was found are indicated in Table I.

Two forms are met with in cultures of this type: (1) rosettes of round, oval, or spindle-shaped cells; (2) extremely slender, long, wavy, darting forms. These will be described as "*spirochetes*," since they correspond exactly to what Schaudinn has designated by that name in connection with his work upon the "leucocytozoön" *H. Ziemanni.* The relative abundance of the two forms varies somewhat in the different strains. Thus, in some the rosette form is very abundant from the start, and the spirochetes are rather scarce, whereas in others the latter appear first and in large numbers, while the rosettes are few and small. Again, in some strains, the spirochetes appear to be much longer than in others. These differences we are inclined to look upon

at present as variations in the several strains rather than as indicative of separate species.

Rosettes.— (See Plates 8 and 9.) In the early stage the rosette consists of a small number of round bodies which may show a few minute granules. They are about 5 μ in diameter and show very little or no motion. They increase rapidly in numbers by means of longitudinal division, giving rise to aggregates of hundreds of cells. Frequently rosettes are met with which, in the somewhat flattened condition due to the pressure of the cover-glass, fill the entire field of a No. 7 Leitz objective.

Eventually, the round or pear-shaped bodies elongate to form ovals, and finally spindle-shaped bodies. At this stage the individuals may be seen to possess a slight swaying motion. The smaller rosettes, consisting of 10–20 cells, measure 14–20 μ in diameter. The spindle-shaped cells are about 10 μ long and about 3 μ wide. They do not apparently break loose from the group, but remain attached. When the growth has reached its full age the spindles begin to form the spherical involutions mentioned above.

It will be seen that the ordinary rosette corresponds in diameter to twice the length of the spindle-shaped cell, that is to say, about 20 μ. The very large rosettes really consist of a number of such smaller ones which can be easily made out in the living condition. The latter may be spoken of as simple or primary and the former as multiple rosettes.

Preparations stained by the Romanowsky method reveal details which cannot be satisfactorily established otherwise. In the first place, the flagella, as in the case of the rosettes of *Tr. Lewisi*, are found to be directed centrally. The relatively large blepharoplast, in an achromic area, is by the side of, or anterior to, the nucleus. This necessarily means that the undulating membrane is very short or even rudimentary, and this explains the absence of motion in the round or oval forms and the slight motion seen in the more elongated bodies. It would appear from this fact that the active motility of the free trypanosome is due largely, if not wholly, to the undulating membrane, and that the free flagellum is incapable of moving or propelling the body.

Furthermore, the stained preparation throws some light upon the formation of the rosette. It appears that they originate in much the same way as the segmentation form of *Tr. Lewisi* in the blood, described by MacNeal[61] and others. In very young cultures it can be seen that the large, free, spindle-shaped trypanosome shortens to form an oval or pear-shaped body, while at the same time the whip is retracted or absorbed (See Plate 9.) The blepharoplast and the nucleus then divide, giving rise to a pear-shaped body with two nuclei and two blepharoplasts. The cell itself may then divide longitudinally, forming two small cells; or, the division of the nuclei and blepharoplasts may be repeated, resulting in a cell with four each of these bodies. In the case where two cells form by the division of the original, they remain attached for some reason by their rudimentary whips, and, as the process of division is repeated, eventually the typical rosette forms. These groups therefore originate by the consecutive division *in situ* of a single cell, and for that reason the multiplication rosette must not be confounded with agglutination masses. The young forms resulting from the division of the oval cell, especially when the consecutive division is rapid, are quite small, about 5 μ long and $2\frac{1}{2}$ μ wide.

The content of the spindle-shaped cells comprising the rosette stains a deeper blue than does that of the spirochete form. The nucleus is always round, large, and shows eight chromosomes. At times a few small colorless granules and globules are present in the cell. These, however, are insignificant in size when compared with similar bodies found in *Tr. Brucei*, *Tr. Mesnili*, and the other types mentioned later.

Spirochetes.—(See Plates 10 and 11.) These are extremely interesting on account of their very rapid motion and their delicate appearance. As seen in the living preparation they appear as long, slender threads, straight or wavy in outline. They are ordinarily single and dart through the field at great speed. Usually, a very fine whip may be seen at one end, but, at times, this may be so delicate as to be invisible. They frequently attach themselves by means of their whip to red blood cells, which are then rapidly pushed through the field. The spiro-

chete, as it travels with the whip foremost, may suddenly stop and move backwards for a short distance.

Dividing forms in the young culture can be observed. The division takes place while the cell is in active motion and without any shortening or rounding up as in the case of the rosette type. The stained preparations are especially useful to bring out this condition. The two young cells resulting from division may remain attached for some time at the posterior end, and, in that case, may simulate an agglutinated pair. The two cells, however, are not in a straight line, but form an acute angle, or they may twist about each other in a spiral manner. Each of the cells, while still attached, may undergo division, thus giving rise to a four-celled group.

The spirochetes show a marked tendency to agglutinate. This begins with two cells sticking together at their posterior ends which slightly overlap. (See Figs. 3 and 4, Plate 10.) In the living preparation the line of junction can not be made out, and as a result the double cell appears to be a single organism with one whip at each end.

A third cell may attach itself by its posterior end to the agglutinated pair, in which case the three bodies may be fairly equidistant. Finally more cells come in and join in the same way, eventually giving rise to tangles of hundreds and even thousands of cells (Fig. 5, Plate 11). We have observed masses of these slender, writhing forms fill the entire field of a No. 7 objective. In these groups or masses the whips are always on the outside or periphery, whereas in the rosettes described above the whips are always on the inside.

It will be seen that the two kinds of groups met with in cultures of this organism are of entirely different origin. The multiplication rosette, with its whips directed centrally, arises by the consecutive division of its cells *in situ*, and is in nowise to be looked upon as an agglutination. On the other hand the spirochetes are normally free and divide while in that condition. These free cells agglutinate in some cultures more readily than in others, in which case the cells join at their posterior ends. The very large rosette always shows the constituent groups of

which it is composed, whereas the agglutinated mass of spirochetes, is without any such regularity.

As the cultures age the spirochetes lose their even form and show a globular enlargement at the posterior end or near the middle. This spherulation may continue until the long form is replaced by a small round body which usually is indicative of the death of the cell. Death may, however, occur without this transformation being completed.

The width of the spirochete in the living condition is about 0.5 μ. The stained preparation, perhaps as a result of flattening, shows the width to be about 1.0 μ. The average length of the spirochete is about 30 μ, but it is not uncommon to meet with cells 50 and even 60 μ long (Plate 10, Fig. 2). The body tapers at each end to a sharp point.

The stain shows that the free flagellum is relatively very short since it measures only about six μ. The blepharoplast, which stains more readily than either the nucleus or whip, is a small dot about 0.5 μ in size. Unlike that of the spindles of the rosette form, it is situated between the nucleus and the posterior end, and distant from the latter by about one-third the length of the body. That is to say, with the body length of 30 μ the blepharoplast is 10 μ from the posterior end. Consequently, the undulating membrane extends along two-thirds the length of this slender body. This explains the extreme motility of this type. Moreover, the shortness of the free whip indicates that it has very little to do with the motion of the cell, as has been pointed out in connection with the rosette form. The position of the blepharoplast, posterior to the nucleus, is an important characteristic, and is not seen in the other species.

The nucleus lies in the anterior half of the body and is approximately of the same width as the cell. In the average spirochete it measures 1×3 μ, while in the very long ones it may be 1×5 μ. When the cell is about to divide the blepharoplast approaches and almost touches the nucleus. At the same time the nucleus becomes round and the cell widens somewhat.

The contents of the spirochete are usually perfectly homogeneous, and in the stains only a few minute granules can be made out.

Owing to the marked difference between the rosette and spirochete forms it may be supposed that they represent two distinct species. This view, however, is untenable since these two forms are present in every one of the 18 strains. Moreover, at no time have we found a culture which had either form by itself, as could well be expected, if they were distinct species. Again, *Tr. Lewisi* in culture presents essentially the same two types. It has multiplication rosettes and free-swimming forms corresponding to the spirochetes, though much wider. This is the case also with several of the other species, as will be shown presently. In our opinion there can be no doubt but that the two forms belong to one species.

It will be remembered that the blood of the birds from which this type was obtained had, in several instances, two forms of trypanosomes—the small spindle—and the long S-shaped trypanosomes. The question naturally arises as to what relation exists between the two forms in the blood and the two forms in the cultures. It is possible that the large S-form is the source of the rosette and that these represent the female cell, while on the other hand the spindle-shaped trypanosome of the blood gives rise to the spirochete which may be considered as an indifferent, or asexual, form. While the latter are capable of multiplying rapidly, they probably do not change into the other type. On the other hand it would seem as if some of the rosette cells could differentiate into the free form. Such a transformation, however, has not been observed.

Sub-type 1a. This can be easily confounded with the preceding. A close examination however, shows that there are some marked differences. The rosettes appear to predominate, and by their number and size attract attention much as they do in the case of *Tr. Lewisi*. The free-swimming forms are much shorter than those of the preceding type, and are distinctly wider near the anterior end. This gives them a slight tapering appearance. They are very actively motile, have clear contents, and agglutinate by their posterior ends. They may be regarded as intermediate between Type 1 and the following, especially Type 4. This type was met with in strains A, B, U, and Z.

Type 2. This was met with but once and then in the blood of a hawk. We have named this species, as already stated, *Tr. Mesnili.* Not only is the trypanosome as found in the blood different from *Tr. avium*, but an even greater difference can be seen in the cultures.

Cultures made from the heart-blood showed on the third day several groups of spherical or oval bodies which measured five to eight μ in diameter. They contained a hyaline plasma, somewhat vacuolated and very highly granular, with numerous bright refractile globules of a greenish tint. These globules were about 0.5 μ wide. The cells composing this group showed a long free flagellum on the outside of the mass. A wide, slowly waving, undulating membrane could also be seen. In addition to this group a number of free, motile, coarsely granular trypanosomes, about 16 μ long, were observed.

On the sixth day the culture was fairly rich, and presented a very striking appearance. The free, swarming forms were numerous, and traveled about very rapidly, whip foremost, at times rolling on their long axis. The body of the cell was from 18–21 μ long and about three μ wide. The greatest width was near the anterior end which narrowed rather abruptly and terminated in a long free whip, From the point of greatest width the body tapered gradually toward the posterior end which was either pointed or slightly blunt. Numerous bright globules or refractive bodies filled the posterior two-thirds of the body. Large, rounded, rolling forms are also common.

The actively motile, free forms showed a tendency to agglutinate in a manner highly suggestive of *Tr. Brucei.* That is to say, the cells adhered not so much by their posterior extremities, but by their sides, thus forming irregular groups of 6–10 or 20 cells. As with *Tr. Brucei*, these agglutinated cells have their whips on the outside (Fig. 4, Plate 5). Frequently they attach themselves to red blood cells.

In addition to the agglutination groups, real multiplication rosettes are to be seen, though rather few in number. These, as in the case of *Tr. Lewisi* and *Tr. avium*, have their flagella on the inside (See Figs. 2 and 3, Plate 5). The older rosettes present

a very characteristic appearance. The spindle-shaped or pyriform cells are on the periphery, while the central portion, 10 to 15 μ in diameter, shows a tangle of slowly waving flagella.

Further details regarding this species will be given under *Tr. Mesnili.*

Type 3.—This was found in a goldfinch, No. 353, in the stained blood of which only one trypanosome could be detected. As seen from Table II the measurements of this cell correspond closely with those of the small form of *Tr. avium*, so much so that if this were the only criterion there would be no hesitation in regarding it as of that species. However, the coarse granulation of the contents, as seen in Fig. 1, Plate 6, suggests a difference, and this is clearly substantiated by the results of cultivation. In view of the fact that this type is so clearly distinct from either *Tr. avium* or *Tr. Mesnili*, we must look upon it as a new species, and have named it, as already mentioned, *Tr. Laverani*, in honor of Dr. Laveran of the Pasteur Institute.

This trypanosome is characterized by a very slow growth in cultures. In such the flagellates are scarcely to be seen before the fifth day; they are moderately abundant about the tenth or twelfth day, and cannot be said to be rich until about the second or third week. Thus, the blood of the bird was planted on two tubes of the medium. Ten days later, each tube showed several rosettes and some actively motile trypanosomes. The maximum of growth was reached shortly before the twenty-third day, for at that time the growth was quite rich, but the motion had disappeared or nearly so. An examination on the thirty-fifth day showed masses of round bodies, many of which were also single. Tangles of free flagella were also common.

The slow growth in cultures, of *Tr. Laverani* distinguishes it at once from either of the preceding types. There are, however, other equally striking differences.

The rosettes are less common than in Type 2 and are perhaps smaller. They are composed of rounded, pyriform, or spindle-shaped cells which contain numerous bright yellowish-green globules, which are larger, and perhaps more abundant, than those of the preceding species. The cells taken as a whole are narrower

than those of *Tr. Mesnili.* Their flagella, directed centrally, are more delicate and hence more difficult to see in the living preparation than are those of the preceding type (Fig. 3, Plate 7).

The free forms, while common enough, are much less motile than is the case with either of the preceding. It may often happen that in a preparation not more than a dozen cells can be found to travel about actively. When moving about they roll on their long axis, whip forward. Usually they attach themselves by their whips to the glass and show a slow swaying motion.

The absence of marked motion is apparently due to a stickiness of the exterior of the free cell. This is seen in the fact that a red blood corpuscle often adheres to the middle portion of the cell when touched by the latter. It is further indicated by the marked tendency to form agglutination groups, which are somewhat suggestive of those of *Tr. Brucei.* The two cells may adhere laterally, with the whips at opposite ends, and the group thus started may increase in numbers so that eventually several hundred cells may be together. In such groups the whips are on the outside and in active motion. The cells usually contain from 5–10 large globules which are found in the posterior end though not always.

Rounded, rolling forms about the size of a red blood corpuscle (Fig. 6, Plate 7) are common in the early cultures and represent dividing forms. In old cultures, the rounding up, as with other trypanosomes, is a common involution change.

The stained preparations show a coarsely granular nucleus, and close to it or anterior is a small blepharoplast. A most interesting feature is the presence of a terminal rod which we have not observed thus far with certainty in any other type. This rod usually lies right up against the wall at the posterior tip of the cell, and for that reason can be easily overlooked. In such a case, all that can be observed is a slightly deeper stain of the edge of the cell at that point. When, however, cell-division is taking place and especially if the preparation is thoroughly flattened out in the act of spreading, this body can be readily seen (Plate 6). That this terminal rod is a distinct structure cannot be doubted. It divides about the same time or a little later than does the

nucleus, but does not approach the latter except possibly in the rounded forms. It is stained a lighter pink by the Romanowsky method than is the nucleus. Thus far we have been unable to make out any connection with the other structures of the cell. It is possible that a similar body is present at the anterior end, as suggested by some of the photographs.

In a recent paper on the development of Herpetomonas, Prowazek[75] describes a flat, spiral thread which passes from the blepharoplast to the posterior end of the body where it terminates in an "undeutlichen Doppelkorn." In the process of reduction previous to copulation this diplosome was said to divide, giving rise to a group of four granules, two of which divided again, so that a group of six granules resulted. The further changes of these bodies could not be followed.

As stated above we have looked closely for some connection with the other structures of the cell, but have not been able to observe any. Possibly, by giving special attention to the method of staining, something may be ascertained on this point. In the dividing cell two such rods are found, but whether the division occurs transversely or longitudinally cannot be said. This terminal rod can be made out more or less easily in the majority of the cells. There are some, however, in which for some reason this cannot be done.

The staining of the cultures of this type is much more difficult than of either of the preceding. The globules which are seen in the living cell do not readily stain, and hence may appear as colorless bodies or vacuoles. On allowing the stain to act for a longer time the globules may be stained a dark red. but in that case the nucleus, blepharoplast, and terminal rod cannot be made out in the deeply stained contents.

Sub-type 3a.—This was obtained from a brown thrasher, No. 386, and is designated as strain T. It is certainly closely related to, if not identical with, *Tr. Laverani.* Like the latter it is a slow grower, requiring from 12–14 days to yield a fair culture. Rosettes are common and like the preceding are made up of round bodies or spindles containing many large globules. Free round bodies 8–10μ in diameter are to be seen rolling about in

early cultures. The free forms 15–18 μ long, unlike those of the preceding, have a narrow posterior end, are narrower and more tapering, and possibly more coarsely granular; they have a smooth gliding motion somewhat circular in course. They apparently are also quite sticky, since they tend to stick to the glass or to agglutinate in groups of 10–20 or more cells. The agglutination is perhaps not as marked as in the case of *Tr. Laverani.*

The length of the body of the free form is on an average 15–18 μ; at times cells 25 μ long are met with. The width varies from 2.5–4 μ. The free whip is from 5–10 μ long, but in the case of the round form it may be much longer. The blepharoplast is very small, usually not over 0.3 μ, and is anterior to the nucleus. The posterior end tapers to a point, whereas the anterior end is commonly blunt. The contents, especially in the anterior half, are rich in granules which stain deeply.

The round forms are, as a rule, about 6 μ in diameter. The smaller ones on division may give rise to cells that measure but $3 \times 5\,\mu$. These usually have a relatively long whip, 10–15 μ long. There is a faint suggestion of a terminal body, as in the case of *Tr. Laverani*, but further staining will be necessary to positively demonstrate this structure. The nucleus is apparently more difficult to stain than is that of the other types.

Type 4.—This was met with three times, and in cultures only. It was obtained twice from blue jays (Nos. 273 and 429), strains M and C′; and once from a rusty blackbird (No. 420) strain A′. In these cases the blood preparations, even on re-examination, failed to show the native trypanosome.

The cultural characteristics of the three strains were practically identical, and for that reason they may be taken to represent a single species. On the other hand, they present marked differences from the preceding types, sufficiently so, to entitle them to be considered as representing a distinct species.

The growth is very rapid, and as a result it is quite rich on the fourth or fifth, and reaches its maximum on about the eighth day. It shows the usual two forms—rosettes and wide, free-swimming cells.

The rosettes are by no means as marked as with the other types. They are scarce and small, rarely consisting of more than about 20 cells. They may be composed of round bodies or of elongated spindles. The whips are on the inside. The spindle forms sway quite freely, sufficiently so as to detach themselves, and it is probably due to this that the rosettes are so few and small. The round bodies and spindles show only a fine granulation of the contents; and are thus in marked contrast to the two preceding types. The round bodies are not much larger than a red blood cell.

The free forms are numerous and move about very rapidly, whip forward. When in motion the cell turns on its long axis, and as a result it may have a wave-like appearance or lateral sway. The body is usually about 15 μ long, and is widest near the anterior end. The width is 3 μ or less. The posterior half may contain numerous small granules, unlike the large globules of *Tr. Mesnili* and *Tr. Laverani*. The undulating membrane can be readily seen at the anterior end.

Agglutination occurs as with the former two types by several cells sticking together lengthwise, the whips remaining on the outside. Large masses of cells may thus form. That this is largely due to a stickiness of the wall is shown by the readiness with which they attach themselves to red blood corpuscles. At times a single cell may be seen to travel about with two such corpuscles.

Sub-type 4a.—This is represented by strain X from goldfinch No. 354. It resembles the preceding but shows considerable variation. The rosettes are even scarcer and are rarely composed of more than a dozen cells which sway actively.

The free form is about 20 μ long and 3 μ wide. Smaller forms of 14–16 μ and larger ones of 24–26 μ are also present. The free whip is from 14–22 μ long. The relatively large blepharoplast (1–1.3 μ) is almost always on the side of the nucleus which is about 2 μ wide. This position of the blepharoplast and its large size distinguishes the cell at once for *Tr. Laverani*. The posterior part of the body is blunt, while the anterior part elongates along the whip to a fine point.

DESCRIPTION OF SPECIES.

Before taking up the description of the species met with by us it will be well to consider briefly the several forms found by other observers and endeavor to correlate their findings as far as possible with our own. This is obviously a matter of some difficulty, owing to the entire absence of cultural characteristics.

The earliest observations, those of Danilewsky, indicated the presence of two forms of trypanosomes in the blood of birds, the small and the large form. As shown heretofore, Danilewsky was undoubtedly correct in the general assumption that these represented but one species, his *Trypanosoma avium*. The close agreement in form and size of his organisms with the majority of the trypanosomes studied by us leads us to accept them as identical, and for that reason we have designated our common species by the old name.

The description of *Tr. avium* as given by Laveran and applied to the organism found in an owl can, in the main, be reconciled with the above. The length, including the whip, is given as 33–45 μ. This, it will be seen, corresponds to the smaller forms mentioned, which measure, without the whip, 20–25 μ. The position of the centrosome, near the posterior end, accords better for the small form than for the large one.

The form which Hanna met with in the blood of pigeons, in India, certainly resembles very closely the large type, such as is shown in Plate 3, and may therefore be looked upon as likewise falling under *Tr. avium*. The same may be said of the trypanosome which he describes as present in the Indian crow, but which Dutton and Todd apparently refer to as in a blue jay.

The short, broad trypanosome which Dutton and Todd found in Senegambia is in form, size, and position of the centrosome the same as the small forms found by us, as will be seen on comparing our photographs with their illustration. It can therefore be regarded as *Tr. avium*.

The *Tr. Johnstoni* of Dutton and Todd certainly represents a distinct species. We have found one single specimen, in a stained preparation of the blood of the woodpecker, No. 391, which resembled somewhat this species. Unfortunately the slide

was misplaced, and as no cultures were made we are unable to give any detail.

The most recent species of bird trypanosome is the *Tr. paddæ*, described by Laveran and Mesnil, and by Thiroux. Were it not for the fact that this organism is said to have a very short free whip, it might well be taken to correspond with the large S-form which is finely striated, as shown in Plate 3. It is to be hoped that cultures of this organism will be made, in which case it will be possible to make an exact comparison with those of ours.*

Trypanosoma Avium, Dan., N. & MacN. Emend.

This is apparently the most common species found in birds, having been met with twenty-five times in the blackbird, bluebird, blue jay, oriole, robin, English sparrow, and song sparrow. This does not include the sub-type 1*a*, previously mentioned, which was found four times, namely, in the mourning dove, blue jay, and flicker.

The native trypanosome may appear in either the small or the large form, or in both. These two forms have already been described, and for that reason, in order to avoid repetition, the reader is referred to that portion of the paper.

It grows very readily on the blood agar medium, and, as a rule, rich cultures are obtained on the fourth to the sixth day. The culture is characterized by the presence of two strikingly different forms, the multiplication rosettes and the free-swimming, darting, thread-like spirochetes.

The rosettes are made up of smaller cells and are a more prominent feature of the culture of *Tr. avium* than are the like formations in the case of the other species. The long, slender spirochete form is apparently distinctive, since the corresponding free-swimming forms of the other species are shorter and wider. Moreover, the blepharoplast in the latter is by the side of or

*As this paper is going to press we learn from Dr. Thiroux that he has succeeded in cultivating this trypanosome which is now in its third generation. An examination of some preparations made from these cultures and kindly sent to us by him show at a glance that this organism is entirely distinct from our Tr. avium. The cultural forms more nearly approach those of Tr. Laverani. Dr. Thiroux has also shown that the injection of Tr. paddae into canaries and paddas is not followed by a halteridium infection and hence that these two organisms are entirely distinct, thus confirming our results and the view expressed at head of page 273.

anterior to the nucleus, whereas in the spirochete it is considerably posterior to this structure.

An extended description of these two forms is given on pp. 282 and 283.

The attempts at infection of birds with cultures of this trypanosome were far from satisfactory. It should be stated, however, that most of these inoculations were made with the object of finding intracellular parasites, which according to Schaudinn's views should be expected, and consequently the search for the trypanosomes was not as thorough as it might have been. The culture fluid used for the injections was either derived from a single strain, or in some instances a mixture of several was used. The injections were as a rule intrapleural, and the fluid was always extremely rich in trypanosomes. The usual dose was from 0.1–0.2 c.c. One screech owl, two pigeons (squabs), three chickens (ten days old), seven robins, twenty-two sparrows, and six old canaries were used. The work with robins and sparrows was rather unsatisfactory on account of the difficulty of keeping the birds alive for any length of time. In only one of these birds, a canary, was a positive result obtained.

This canary received an injection of a very rich culture of strain D. Four days later, it showed a marked leucocytosis, and three trypanosomes were found in the fresh blood. These were in the form of a long, straight spindle, about 4.5 μ wide and 28 μ long, with a free whip measuring 12–15 μ. The undulating membrane was very conspicuous, starting from near the posterior end and passing over the body in two large waves. The nucleus was readily visible as a large oval, about as wide as the parasite. Fine granules were present, especially in the posterior part. The motion was rather sluggish and confined to the neighborhood of the one field of the microscope. The next two examinations, made about a week apart, were negative. On the third and fourth week a single trypanosome was found each time. It then appeared to be smaller than on the previous occasion. The body was about 17 μ long, three to four μ wide, and the whip measured about eight μ. Three examinations in the course of the next two weeks were again negative. The trypanosomes were thus

detected, off and on, for eleven weeks, and were found in the blood at the time of death. Although many attempts were made to obtain stained preparations of the trypanosome, they all failed, owing to the extreme scarcity of the parasite. Immediately after death cultures were made from the heart-blood of this canary, and in four days they showed a rich growth, consisting of the typical rosettes and spirochetes of *Tr. avium*. This culture, known as strain Y, is not included in Table I, since it was derived from an artificially infected bird. It makes the thirtieth strain isolated from birds.

Trypanosoma Mesnili, N. Sp.

This trypanosome was found in the blood of a red-shouldered hawk (*Buteo lineatus*), associated with *halteridium* and *H. Ziemanni*. The organism was very scarce, since only one specimen could be found on four slides, each of which was systematically searched by means of a movable stage.

The native trypanosome (see Fig. 1, Plate 5) is characterized by its large size and great bulk, and by a wide, rounded posterior extremity. The length of the body is 50 μ, and the width opposite the nucleus is 8 μ. The width of the cell at the posterior end is 5 μ. The distance from the posterior end to the centrosome is 7 μ, and from this to the nucleus it is 10 μ. The anterior portion of the body, which is bordered by a thick, undulating membrane, tapers rather abruptly to a narrow end which undoubtedly terminates in a free flagellum. This, however, cannot be made out in the specimen. The trypanosome was not seen alive in the blood.

The contents of the cell stain heavily and are coarsely granular. The centrosome is round and about 1 μ wide. The nucleus is relatively small and is found near the side having the undulating membrane.

The cultures of this organism grow very rapidly, and are very rich on the sixth to the seventh day. After that they undergo involution changes, forming large masses of very granular, round bodies. A few motile cells may be met with in such cultures, even at the end of six weeks.

The cultures show two types of cells, resembling in a general

way those met with in *Tr. Lewisi* and *Tr. avium*. The one type makes up the multiplication rosette, while the other is free and swarming, and corresponds to the spirochete stage of *Tr. avium*.

The multiplication rosettes (see Figs. 2 and 3, Plate 5) vary considerably in size. The smaller ones are about 10 μ in diameter, and are made up of cells in process of active division. Such cells are about 3 μ wide and 5–6 μ long, and on division yield cells which are about 2 μ wide and 4 μ long. On account of the rapid division, the free flagella are very short and can scarcely be made out.

The larger rosettes consist of more fully developed cells, 10–100 in number. These are usually about 10 μ in length, but they may attain a width of 6 or even 7.5 μ. The full-sized spindles measure 4×15 μ. The free whips, about as long as the cell, are easily seen in the central portion of the rosette. On account of the length of the whips the cells composing such rosettes exhibit a marked swaying motion. The large oval or pyriform cells stain deeply, and are filled with dark granules and bright refracting globules. When free, as is often the case, they may be seen rolling about in the fluid.

The free, swarming cells, as stated above, correspond to the spirochete stage of *Tr. avium*. Unlike the spirochetes of the latter, they are short and very wide, and are provided with very long, free flagella. Such free cells ordinarily measure 4–6 μ in width and 20–25 μ in length, not counting the whip, which often measures 17–20 μ. From the widest portion, near the anterior end, the body gradually tapers toward the posterior extremity, which is either blunt or slightly pointed. On account of their large size, the undulating membrane can be seen at the anterior end, and for the same reason these cells have a stately, gliding motion. They travel rapidly, whip foremost, either in a straight line or in a circle, and often may be seen to roll on their long axis.

When the growth is very rapid, as a result of a specially favorable medium, the cells show only very minute globules, scattered all through the plasma. At other times the granules are

rather large, and have a distinct yellowish green color. They are most abundant in the posterior half of the cell.

We have pointed out in connection with our other work on the cultivation of trypanosomes that the presence of bacteria tends to destroy these protozoa. The result is largely dependent on the kind of bacteria which chance to be present. In one instance, Laveran and Mesnil found the culture of *Tr. Lewisi* to remain alive and virulent in the presence of bacteria for about two weeks. A similar result, extending over a much longer period of time, we have noted in connection with *Tr. Mesnili*. A culture of this organism developed a contamination with a slow-growing streptococcus, and inasmuch as the trypanosome continued to grow, even luxuriantly, it was transplanted and subcultures were kept up. These have now been maintained for over three months, and, if anything, the mixed cultures are somewhat better than the pure ones.

The free, swarming trypanosomes show a considerable tendency to agglutination. This usually begins by two cells overlapping at their posterior ends. Other cells attach themselves to these by their sides, and thus large masses soon form. At times this agglutination takes place so rapidly under the cover-glass that, in the course of an hour or two, nearly all of the cells gather into these irregular clumps. In such agglutination groups the flagella are always on the outside. The undulating membranes and whips continue their motion, and as a result the entire mass can be seen to travel through the field.

The fact that the cells agglutinate along their whole length would seem to indicate that the surface of the body is more or less sticky. This view is substantiated by the behavior of these cells with respect to blood corpuscles. In some cultures it is a common occurrence to find the trypanosomes moving rapidly about with a red blood cell attached to the median portion. This could hardly happen unless the outside of the trypanosome was somewhat gelatinous. There is also a tendency, especially for the larger forms, to attach themselves by means of their whip to the glass surface.

The free motile forms can be seen in all stages of division.

For this purpose the cell first shortens to a large pyriform body about 10 μ wide and 15 μ long. The whip likewise shortens. The blepharoplast divides first, then the nucleus, and finally the body itself begins to split (Fig. 5, Plate 5).

Only two injections of cultures of this trypanosome were made, and both were negative. A young screech owl (*Megascops asio*) and three young chickens (10 days old) were used.

TRYPANOSOMA LAVERANI, N. SP.

This interesting species was met with but once and that in goldfinch, No. 353. It was overlooked in the examination of the fresh blood and in the stained preparations. When, however, the culture was obtained from the heart-blood the original stains of the blood were re-examined by the help of a movable stage, with the result that one trypanosome was found on three slide smears.

The native trypanosome is shown in Fig. 1, Plate 6. It is a wide spindle which measures 20 μ in length and 6 μ in width. The blepharoplast is close to the sharp-pointed posterior end, about 1 μ distant. The photograph shows no free whip, but this must be considered as due to defective staining since in the cultures a long free flagellum is easily observed. The posterior part of the cell is coarsely granular which peculiarity is brought out very clearly in the cultures. The wide nucleus occupies the middle of the cell.

In artificial culture the trypanosome is characterized by a very slow and sparse growth.

The multiplication rosettes are rather few in number and are small. They consist of rounded or pyriform bodies, which eventually elongate to oval or spindle-shaped cells. In the living preparation these are found to contain numerous granules and bright yellowish-green highly refracting globules which do not readily take the dye, and consequently in stained preparations appear as colorless globules (see Plate 7). The globules are usually about 1 μ in diameter, but at times they may be found twice that size.

The spherical forms which may be looked upon as giving rise to the rosette are frequently found free and in active division.

The blepharoplast divides first, then the nucleus, and lastly the protoplasm. The division is longitudinal and uneven, that is to say, the young cell may be only half as wide as the remaining part of the parent cell. The latter may therefore pass on to a second or third division, and, as a result, groups of three or four of such cells are often found. Such round or oval bodies usually measure $8 \times 10\mu$, but may attain a size of $12 \times 14\mu$. A noticeable feature in these bodies is the terminal rod which usually lies against the posterior wall, but may at times approach the nucleus. The free whips on these forms are relatively short, about $10–14\mu$.

The free forms appear, either as slender spindles, which have a fairly constant width along the entire length of the body, or as spindles which are appreciably wider in the middle portion and taper toward both ends. The latter are from $14–20\mu$ in length and $4–5\mu$ in width, and have a free whip which is as long or even longer than the body. The posterior end may be sharp but more often is blunt or cut off square, and shows the peculiar terminal rod. The slender spindle, mentioned above, measures from $20–25\mu$ in length and $2.5–3\mu$ in width. The whip is relatively shorter than in the preceding type, measuring about $10–14\mu$. In both forms the blepharoplast which is comparatively small lies anterior to the nucleus. It is usually about 0.7μ long, but in the dividing forms it may attain a length of nearly 2μ.

The free form divides by longitudinal fission without shortening or rounding up to any appreciable extent. The blepharoplast divides first, and this leads to the formation of a new whip and then, very often, to a division of the anterior portion of the cell. The nucleus then divides and the cleavage continues until complete division results. The terminal rod apparently divides after the nucleus, and, at the stage of complete division, two of these bodies can be seen at the posterior end.

The relatively feeble motion and the marked tendency to agglutinate have been mentioned under Type 3.

No inoculation experiments have as yet been made with this culture.

Unnamed Species.

The three strains described under Type 4 without doubt represent a distinct species, but inasmuch as the native trypanosomes

have not been found, even after repeated search of the stained preparations of the blood, it has not seemed desirable at present to give this type a definite name. Inoculation experiments are now in progress and may help to supply this deficiency.

As to the sub-types 1*a*, 3*a*, and 4*a*, the opinion may also be expressed that they belong to new species. It has not, however, been possible up to the present time to give these a very thorough study, especially of the stained preparations of the cultures, and consequently they must be reserved for another occasion.

RELATION OF TRYPANOSOMES TO THE CYTOZOA.

This subject has acquired especial importance in view of the work of Schaudinn on the halteridium and the "leucocytozoön" of Danilewsky. The main conclusions arrived at by this investigator have been given in the introduction, and as already indicated they are not substantiated by our work.

In the first place we have shown that trypanosomatic infection of birds is widespread. Moreover, there is sufficient evidence of the existence of a large number of species of trypanosomes, and, as can be seen in Table I, a given species of bird (bluejay, goldfinch) may be subject to infection by two or three kinds of trypanosomes.

Double, triple, or even quadruple infection in birds is not an uncommon occurrence, and on that account it is not surprising to find trypanosomes associated with one or more intracellular parasites. Such association, however, cannot be said to have any other significance than that of mere chance or accident. The two extremes: first, of cytozoa without trypanosomes; and second, trypanosomes without cytozoa, are common enough as has been shown. Furthermore, it has been made evident that a given cytozoön, as, for example halteridium, may be associated with at least three distinct species, *Tr. avium*, *Tr. Mesnili*, and *Tr. Laverani*. Again, an examination of Table I will show in the case of the robin that the same species, *Tr. avium*, may be associated with any one of four cytozoa. The remarkable uniformity of the occurrence of this species of trypanosome under these conditions is difficult of explanation from the stand-

point taken by Schaudinn. It is also worth noting that in the one case of infection with the "leucocytozoön" *H. Ziemanni* we have obtained a trypanosome, *Tr. Mesnili*, which in cultural characteristics is totally different from the *Spirochete Ziemanni* described by Schaudinn as a stage of this cytozoön.

In view of the ease with which trypanosomes can be cultivated in the test-tube it is reasonable to suppose that the same result can be obtained with the mosquito. That is to say, the few trypanosomes which may chance to be present in the blood sucked up by the mosquito rapidly multiply in the stomach of the insect and give rise to rich cultures similar in every respect to those met with in the test-tube. Thus, the multiplication rosette, with the whips directed centrally, will be found as the predominating feature with a given species of trypanosome. In the case of *Tr. avium* the long, slender, free forms or spirochetes are especially noticeable.

It seems to us, therefore, that the observations of Schaudinn are open to an entirely different interpretation than that given by him. It appears that he has cultivated trypanosomes *in vivo* and has obtained forms which agree fully with those obtained by us in artificial culture, *in vitro*.

Assuming the identity of the flagellates found by Schaudinn and ourselves *in pure culture*, it can be expected that like results would be obtained by the injections, on the one hand, of suspension of the mosquito culture, and on the other hand of the culture fluid from the test-tube. On this point we have made a very large number of inoculations into sparrows, robins, owls, canaries, young pigeons, ring doves and very young chickens, but have never been able to obtain any indication of the development of intracellular parasites as a result. The amount of culture fluid thus injected into a bird was incomparably greater than could be obtained by the injection of a large number of mosquitoes. It is true that with one exception we failed at the same time to infect the birds with trypanosomes.

Since the injection of suspensions of the infected mosquitoes, or the mere bite of a few of these insects, as shown by Schaudinn and by the Sergents, is capable of causing an infection with the

intracellular parasites, such as the halteridium, it follows that one of two conclusions can be drawn. First, it may be held that the flagellates observed by Schaudinn in the mosquito are the real stages of the cytozoön and that they are wholly distinct from the trypanosomes found in birds. Or, second, it may be held that the flagellates found in the mosquito are derived from the trypanosomes in the birds, and as such have nothing to do with the intracellular parasites; in which case the positive infection with the mosquito must be due to some as yet unrecognized stage of the cytozoön. This latter view is, in our opinion, the correct one.

In order to furnish a definite solution of this question it will be necessary to allow mosquitoes to bite birds which by the cultural method have been shown to be free from trypanosomes. If flagellates appear in the stomach of such mosquitoes, after feeding on birds known to be free from trypanosomes, it will be justifiable perhaps to consider them as stages in the development of cytozoa. On the other hand, since only about 10 per cent of the mosquitoes which feed upon the infected bird develop flagellates (according to the Sergents about 25 per cent), it ought to be possible to produce with those which show no trypanosomes the cytozoön infection.

The recent detection by Rogers[76] of trypanosomes in so-called culture, made with the blood of patients infected with *Piroplasma Donovani* is assumed by some to indicate that the trypanosome is a stage in the development of that parasite. This position, however, is far from being established. It may well be asked, in view of the great frequency of trypanosomatic infection of birds, whether or not a similar condition may not obtain with man, especially in the tropics. The direct detection of such trypanosomes in the blood would be perhaps as difficult of accomplishment as it is in the case of birds, and for that reason they could be easily overlooked. It is probable that if systematic cultivations are made of the blood of a large number of individuals inhabiting the warm countries, conditions will be found to parallel the infection of birds, rats, fish, and amphibians. On *a priori* grounds one could expect to find relatively harmless trypanosomes in the blood of man besides the one pathogenic *Tr. gam-*

biense. The recovery from trypanosomatic infection of the one known case, that of a European, may be looked upon as due to the presence of a different trypanosome from that of sleeping sickness. Similarly, the observation of Rogers may be interpreted as indicating the existence of a hitherto unrecognized form of trypanosomatic infection in man.

SUMMARY.

The main results arrived at in this investigation can be briefly summarized as follows:

1. Trypanosomatic infection of birds is very common and widespread.

2. The different species of birds may harbor different species of trypanosomes; and the same species of bird may be infected by several species of these parasites.

3. The trypanosomes may or may not be associated with intracellular parasites; and no constancy can be shown to exist between a given trypanosome and a given cytozoön.

4. The *Trypanosoma noctuæ* and the *Spirochete Ziemanni* of Schaudinn probably represent trypanosomes which have multiplied in the mosquito; and are not to be considered as stages in the life-history of cytozoa.

5. The unsuspected frequency of trypanosomes in birds makes it probable that, in the tropics, animals and even man may be found to harbor in small numbers such parasites. The presence of trypanosomes in the blood of man does not necessarily mean an infection with *Tr. gambiense*.

In conclusion it is a pleasure to acknowledge the aid given by the Rockefeller Institute in the furtherance of this work. Our thanks are also due to Dr. J. F. Eastwood for his great help in the photographic room, and to Mr. H. N. Torrey, the present holder of the Rockefeller fellowship, for his invaluable assistance in the work of cultivation and staining.

EXPLANATION OF PLATES.

These illustrations were taken by means of the large Zeiss microphotographic apparatus. With the exceptions noted the objects are all magnified 1,500 diameters. They are all stained by the Romanowsky method, except Fig. 5, Plate XI, in which case a living preparation was used.

PLATE 1.—NATIVE TRYPANOSOMES IN THE BLOOD OF BIRDS, *Tr. avium*.

FIG. 1.—Trypanosome giving rise to strain A, in mourning dove, No. 5.

FIG. 2.—The same of strain B, in mourning dove, No. 6.

FIG. 3.—The same of strain C, Baltimore oriole, No. 41.

FIG. 4.—Another slide of the same blood as preceding, showing the crushed form.

FIG. 5.—Trypanosome of which no culture was obtained, from Baltimore oriole, No. 272.

FIG. 6.—Another slide of the same blood as the preceding, showing a slightly crushed form. Note flagellum.

PLATE 2.—NATIVE TRYPANOSOMES IN THE BLOOD OF BIRDS, *Tr. avium*.

FIG. 1.—Trypanosome giving rise to strain G, robin, No. 50.

FIG. 2.—The same of strain D, robin, No. 51.

FIG. 3.—The same of strain F, robin, No. 54.

FIG. 4.—From same preparation as preceding, showing the trypanosome slightly flattened. To the left a cell infected with a female form of *Hæmoproteus majoris*, Lav. This shows in the center a faintly stained nucleus, while adjoining and overlapping it is the large deeply stained blepharoplast.

FIG. 5.—Small form of trypanosome, of which no culture was obtained, from robin, No. 270.

FIG. 6.—Large S-shaped trypanosome in the same preparation as the preceding. The undulating membrane is particularly well developed. The nuclear portion is not stained.

PLATE 3.—NATIVE TRYPANOSOMES IN THE BLOOD OF BIRDS, *Tr. avium*.

FIG. 1.—Small form of trypanosome giving rise to strain K, in blue jay, No. 244.

FIG. 2.—Another preparation of the same blood showing the crushed form.

FIG. 3.—Large trypanosome S-shaped in the same preparation as that of Fig. 1. The blepharoplast, surrounded by achromic zone, is near the nucleus. Note the striations on the surface of the cell.

FIG. 4.—Large form in same preparation as preceding.

FIG. 5.—Small form of trypanosome of strain R, in blue jay, No. 278. Note the free flagellum.

FIG. 6.—Large S-shaped trypanosome in same preparation as preceding.

PLATE 4.—NATIVE TRYPANOSOMES IN THE BLOOD OF BIRDS, *Tr. avium*.

FIG. 1.—Trypanosome giving rise to strain E, in robin, No. 53.

FIG. 2.—The same of strain H, in song sparrow, No. 142.

FIG. 3.—Small form of trypanosome, of which no culture was obtained, in song sparrow, No. 288.

FIG. 4.—Large form in same preparation as preceding.

FIG. 5.—Trypanosome, of which no culture was obtained, in blackbird, No. 401.

FIG. 6.—Another in same preparation as preceding.

PLATE 5.—NATIVE AND CULTURAL FORMS OF *Tr. Mesnili*, n. sp.

FIG. 1.—*Tr. Mesnili* in blood of hawk, No. 350, source of strain P.

FIG. 2.—Culture of *Tr. Mesnili* on blood-agar, gen. 6, five days at 25°. Small multiplication rosette consisting of large and small cells, some in process of division. The flagella are central and short.

FIG. 3.—From same preparation as preceding. Large multiplication rosette with full-grown cells, some dividing. The flagella are long and centrally directed.

FIG. 4.—From same preparation as preceding: agglutination group of five swarming cells. Reduced from 1500 × to about 1160 ×.

FIG. 5.—From same preparation as preceding. A free-swarming form, like that shown in Fig. 4, in process of division. In the center are two faintly stained nuclei while the two blepharoplasts are deeply stained.

PLATE 6.—NATIVE AND CULTURAL FORMS OF *Tr. Laverani*, n. sp.

Note in the latter the presence of the terminal rod at the posterior end; also the smallness of the blepharoplast and its position, anterior to the nucleus.

FIG. 1.—*Tr. Laverani* in blood of goldfinch, No. 353.

FIG. 2.—Free form with blunt end, culture 12 days at 25°, Gen. 6.

FIG. 3.—Free tapering form. From same slide as preceding.

FIG. 4.—The slender or narrow free form. This and the next two are from a culture in Gen. 7, grown six days at 25°.

FIG. 5.—The free form in process of division; note the two nuclei, two blepharoplasts, and two flagella; also the presence of colorless globules.

FIG. 6.—Dividing form, with two nuclei and two blepharoplasts; each of the latter is in process of division as shown by the fact that each gives off two flagella. Note the prominent terminal rods.

PLATE 7.—CULTURAL FORMS OF *Tr. Laverani*.

These were obtained from the seventh generation grown at 25° for 6 days. Note the prominent terminal rod and the position of the blepharoplast, anterior to the nucleus; also the presence of the colorless globules.

FIG. 1.—Dividing free form with one nucleus and two blepharoplasts.

FIG. 2.—The same as Fig. 1, but with two nuclei and two blepharoplasts. Compare this and the preceding figures with Fig. 5, Plate 5, and Fig. 1, Plate 11.

FIG. 3.—Multiplication rosette of pyriform and spindle-shaped cells. Note the tangle of flagella at the center.

FIG. 4.—Large and small oval bodies, the former in process of division. Note that each blepharoplast is giving off two whips, which indicates that another division is about to occur.

FIG. 5.—Like Fig. 4, but the division has gone on resulting in the formation of four nuclei, and four blepharoplasts, and four whips.

FIG. 6.—Earlier stage of the round form than is shown in Figs. 4 and 5. One nucleus, two blepharoplasts, and two whips.

PLATE 8.—CULTURAL FORMS OF *Tr. avium*.

Strain Q from robin, No. 257, gen. 7, grown seven days at 25°. The figures on plates 8, 9, and 10, and figs. 1, 2, 3, 4 of Plate 11 were made from the same slide.

FIG. 1.—Early type of multiplication rosette consisting of round bodies. Shows whips on the inside.

FIG. 2.—Another multiplication rosette, cells oval in form. One spirochete in the field. Whips as before.

FIG. 3.—Another multiplication rosette of short, narrow spindles. Whips as before.

FIG. 4.—Large multiplication rosette of fully developed spindles. Whips as before. Some cells dividing.

FIG. 5.—Small rosette from same preparation as preceding. The two rounded cells are in process of division as seen by the two stalks or whips which each gives off; also by the partial division of the blepharoplast.

PLATE 9.—CULTURAL FORMS OF *Tr. avium.*

From the same preparation as before. The figures show the initial stage of rosette formation.

FIG. 1.—The remnant of whip shows on each cell. The smaller cell has not begun to divide, the larger shows two nuclei and two blepharoplasts; one of the latter is dividing.

FIG. 2.—Large form as before, with four nuclei and five blepharoplasts.

FIG. 3.—Rosette of several such bodies.

FIG. 4.—Two cells resulting from the division of the round form.

FIG. 5.—Early stage of rosette consisting of four cells. Beside it a spirochete.

FIG. 6.—Rounded form, possibly of a spirochete, shows three nuclei and three blepharoplasts deeply stained. The lines on the surface are probably short flagella.

PLATE 10.—CULTURAL FORMS OF *Tr. avium.*

From same preparation as before. The figures show the swarming spirochete type.

FIG. 1.—A group of spirochetes.

FIG. 2.—A very long spirochete (50 μ) and a short one.

FIG. 3.—Agglutination of two spirochetes, showing the overlapping of the posterior ends.

FIG. 4.—Another agglutinated pair of spirochetes; the two cells are almost fused together.

A mass of agglutinated spirochetes is shown in Fig. 5, Plate 11.

PLATE 11.—CULTURAL FORMS OF *Tr. avium.*

From same preparation as before, except in case of Fig. 5.

FIG. 1.—Incomplete division of a spirochete; two separate nuclei while the dark oval blepharoplast shows that it is constricted, if not actually divided.

FIG. 2.—More advanced stage of division of spirochete, showing two nuclei and two blepharoplasts.

FIG. 3.—Complete division of a spirochete. The posterior end of one cell projects beyond that of the other.

FIG. 4.—Rounding up of a spirochete.

FIG. 5.—A mass of agglutinated spirochetes from strain Y. This was obtained from a canary inoculated with strain D. The preparation was living and motile. The photograph was taken by means of instantaneous exposure. Magnification, 500 ×.

PLATE 1.

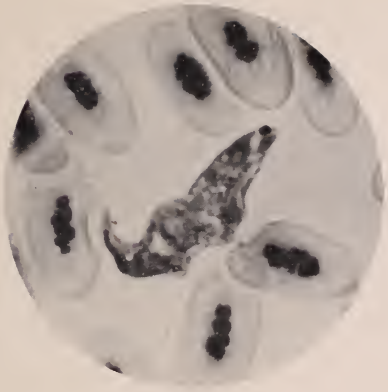


FIG. 1.

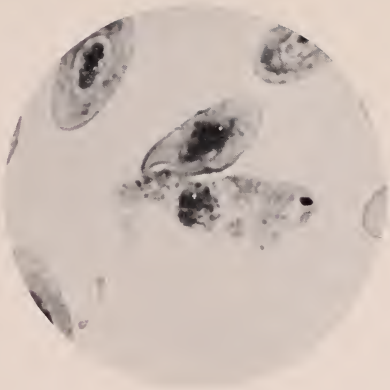


FIG. 2.

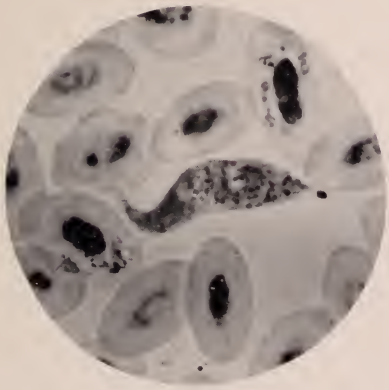


FIG. 3.

FIG. 4.

FIG. 5.

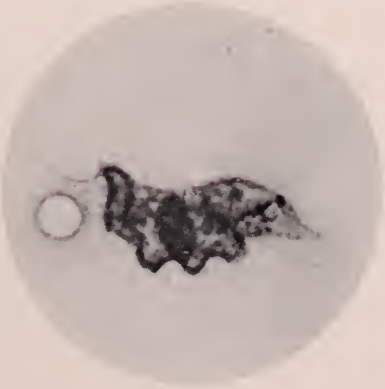


FIG. 6.

PLATE 2.

FIG. 1.

FIG. 2.

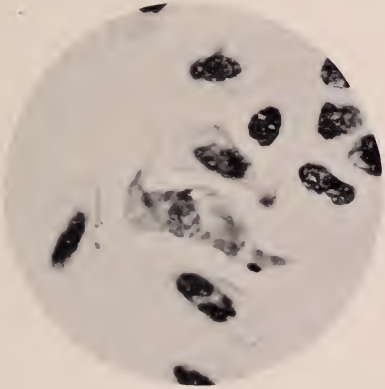


FIG. 3.

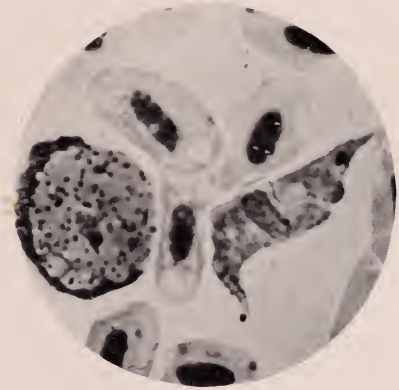
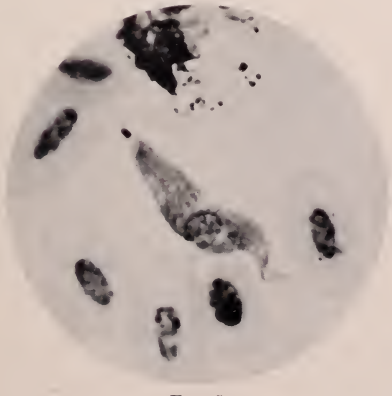


FIG. 4.



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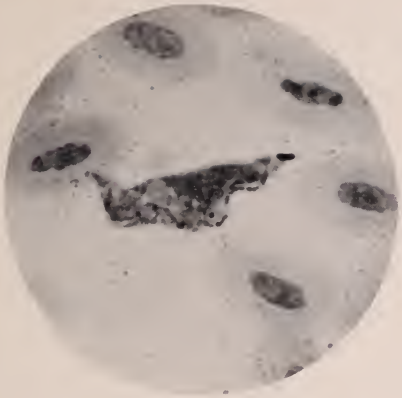

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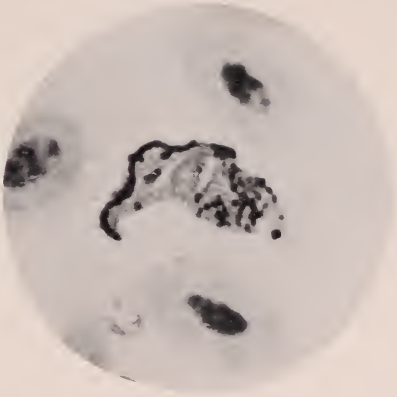

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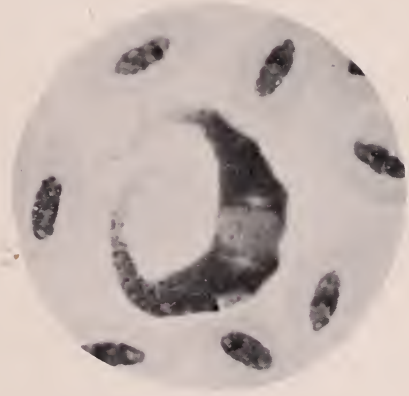

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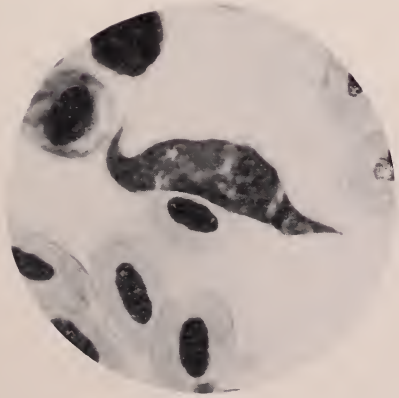

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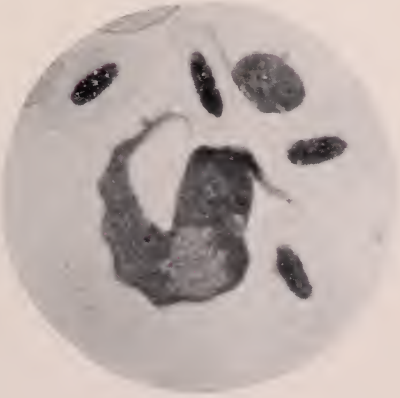

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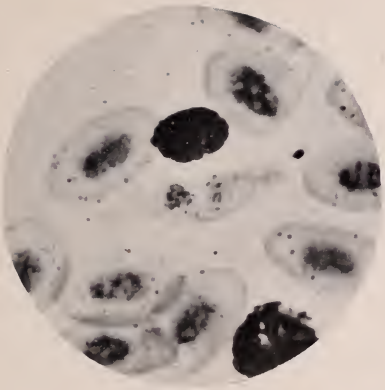

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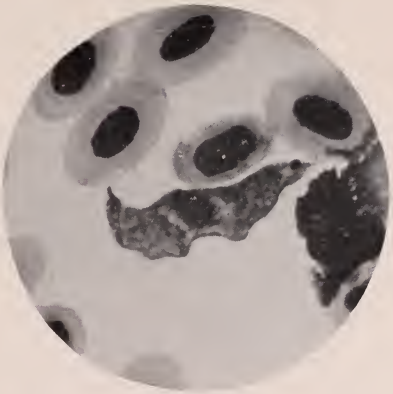

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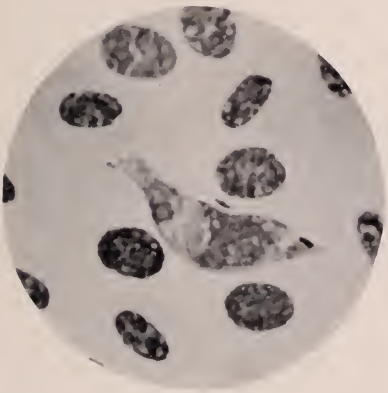

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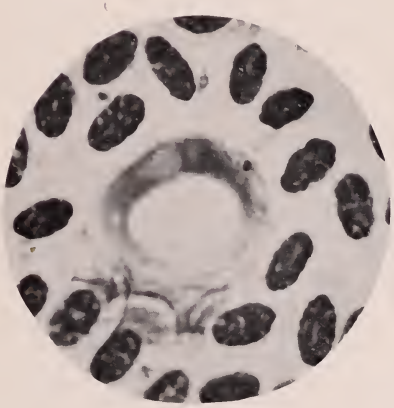

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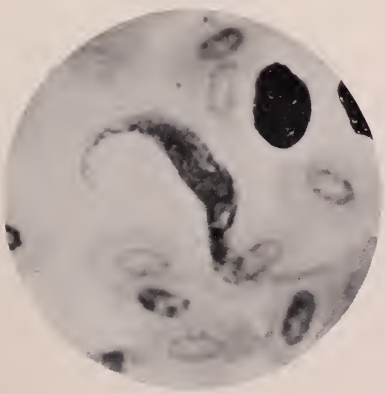

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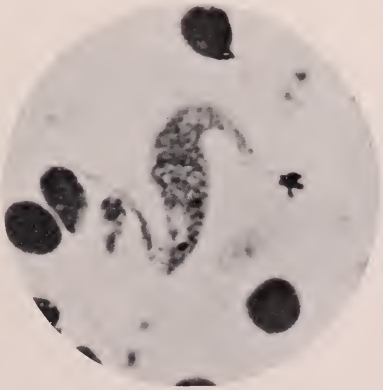

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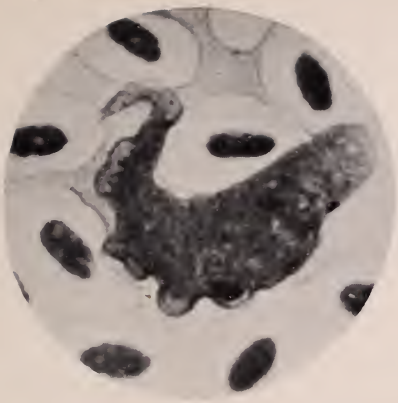

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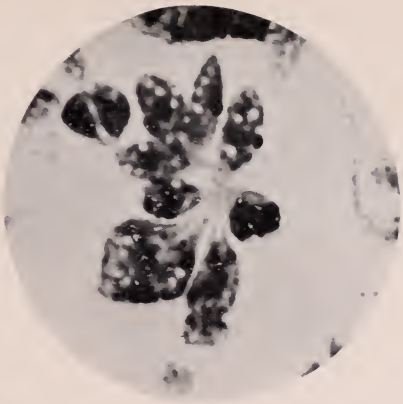

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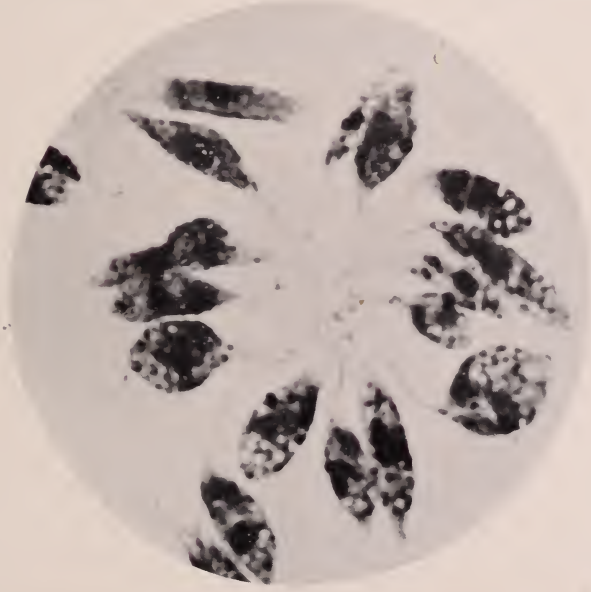

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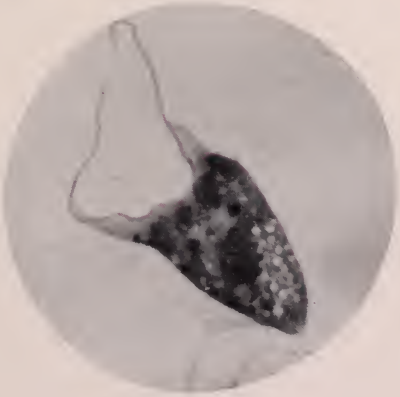

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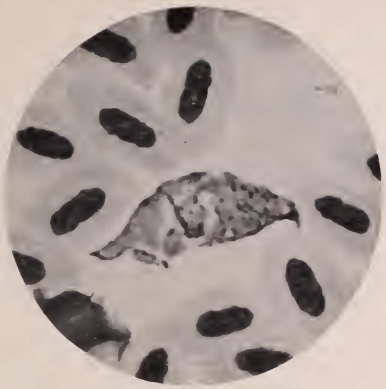

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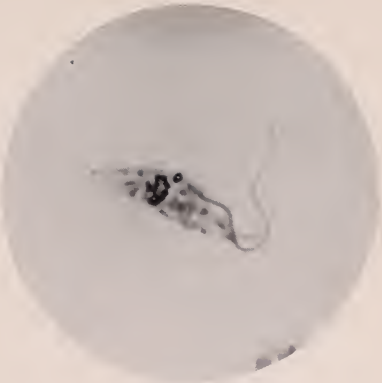

FIG. 2.

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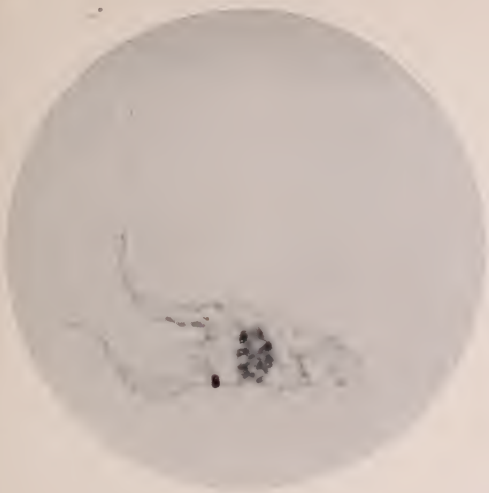
PLATE 7.



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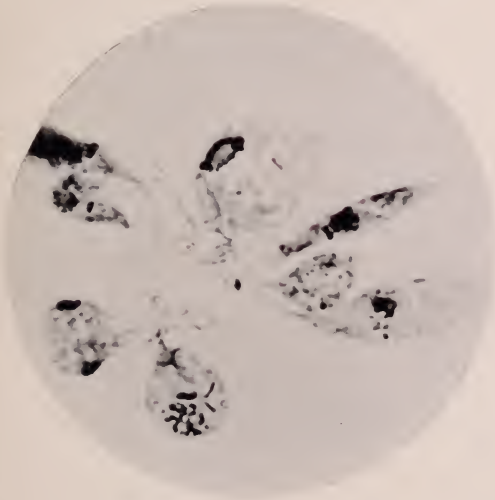

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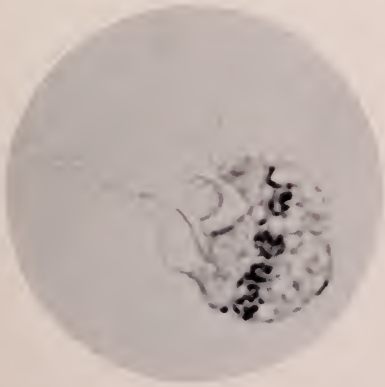

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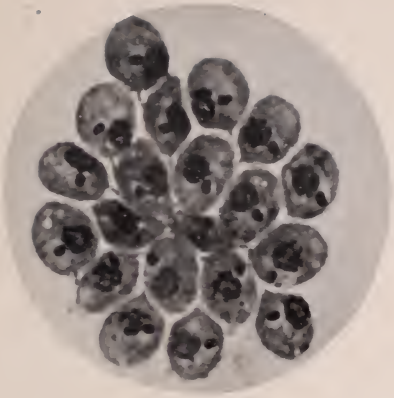
PLATE 8.



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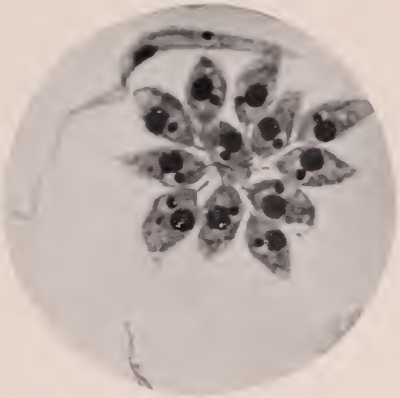

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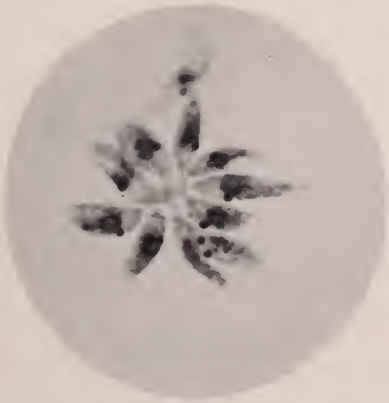

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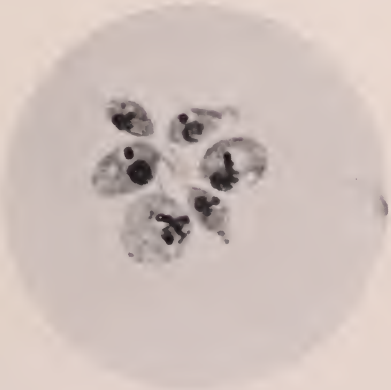

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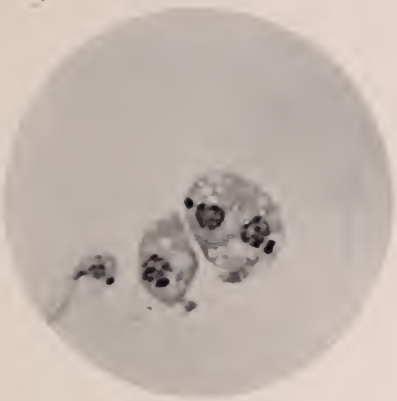

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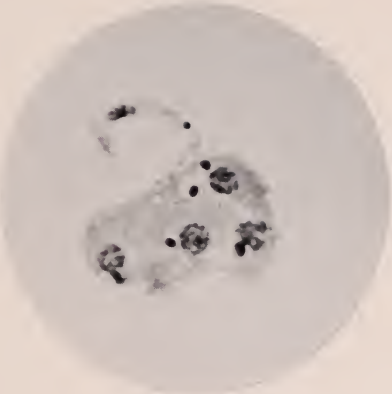

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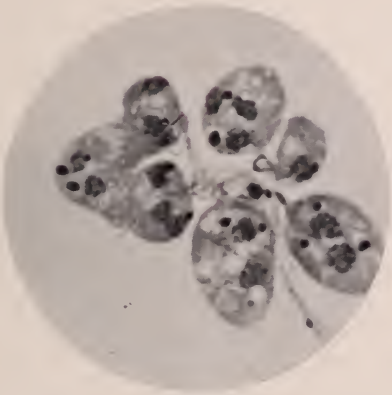

FIG. 3.

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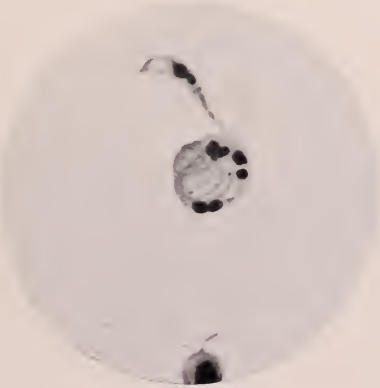

FIG. 6.

PLATE 10.

FIG. 1.

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PLATE 11.

FIG. 1.

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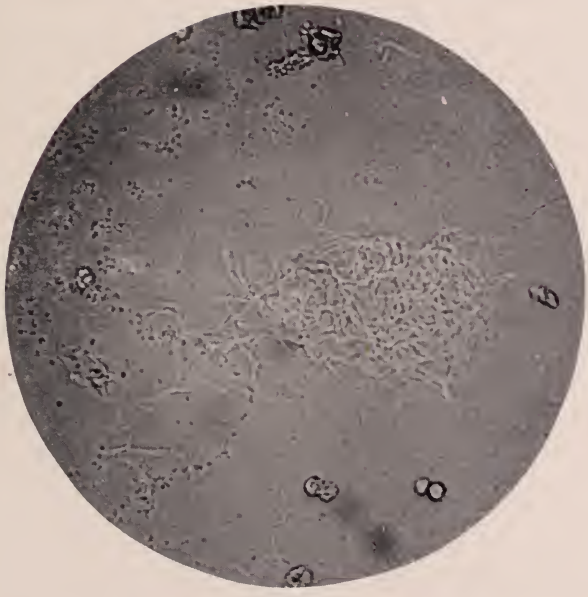

FIG. 5.

FIG. 3.

FIG. 4.

SOME OBSERVATIONS ON THE BIOLOGY OF THE CHOLERA SPIRILLUM.*

WILLIAM B. WHERRY,
Bacteriologist, Biological Laboratory, Manila, P. I.

CONTENTS.

Introduction.
Description of the method of preparing and neutralizing the media.
Source, isolation, biochemical peculiarities, and variations of culture "579," with especial reference to
(*a*) Demonstration of the cholera-red reaction;
(*b*) Liquefaction of gelatin;
(*c*) Optimum reaction;
(*d*) Production of alkali.
Description of the source and isolation of five other cholera cultures and of their resemblance to one another and to culture "579."
Growth in the presence of carbohydrates.
Relationship as shown by agglutinating and bactericidal sera.
Pathogenicity.
Morphology and pleomorphism.
Summary.

INTRODUCTION.

The following observations were made during the past year while I was engaged in some studies in connection with the subject of toxin production.

Such marked variations in the morphology and in the biochemical characters of the cholera spirillum occurred during some earlier work that it was deemed advisable to adopt a modification of the usual methods of standardizing culture media.[1] It is to be regretted, from a purely descriptive standpoint, that the organisms were not grown upon media prepared exactly according to the recommendations of the American committee; but since the main issue concerned the factors influencing toxin production, and since it was impossible to carry on two entirely separate sets of observations, a slight modification of these recommendations was adopted, for reasons which are given below.

*Received for publication December 3, 1904.

[1] "Procedures recommended, etc," Reports and Papers of the *Amer. Pub. Health Assn.*, 1898, 22, p. 60.

Notwithstanding the use of these methods, a comparison of the biochemical peculiarities of the cultures chosen for this study reveals many points of difference between them; *e. g.*, the production of a pellicle on broth by one, while another gives a diffuse cloudiness, the presence or absence of the cholera-red reaction, variations in the growth on potato, or in the type of the liquefaction of gelatin. Many of these points of difference are still emphasized in bacteriological literature, especially in the descriptions of single species. A careful preliminary study of one of these cholera cultures ("579") showed such a wide variation in its morphology and in some of the details of its cultural characteristics that I was forced to the conclusion that they could not be seriously considered in species description, since they are variations which will occur at intervals in the same culture.

It is hoped that this study, which was carried out under more uniform conditions than can be attained by older methods, will emphasize the variability of some bacteria, and in a measure promote investigation of the factors entering into the production of such variations.

DESCRIPTION OF THE METHOD OF PREPARING THE MEDIA.

One of the chief modifications of the methods recommended by the American committee concerns the way in which the media were neutralized and the desired reaction obtained. The recommended method consists, briefly, in titrating a portion of the medium, as near the boiling point as possible, with phenolphthalein as an indicator; in obtaining an accurate neutral point by the addition of normal NaOH to the bulk of the medium, and adding sufficient normal HCl to give the desired reaction. In my work the acidity or the alkalinity to phenolphthalein was adjusted by the addition of normal NaOH alone, and, unless otherwise stated, the "reaction" refers to that established before sterilization.[1]

This modification was adopted for the following reasons: Some broth was prepared in our laboratory in which the cholera spirillum

[1] The term "final reaction" refers to the reaction of the medium after sterilization or just before its use. The + sign stands for acidity, the − sign indicates alkaliuity; thus +1 means one per cent. acid to phenolphthalein, *i. e.*, 10 c.c. of normal acid to 100 c.c. of medium.

would not grow at 35–37°, whereas B. coli grew in it luxuriantly at that temperature. Upon investigation it was found that if, after neutralization with NaOH, the precipitated acid albumins are first filtered out and HCl then added, the acid may exert a germicidal or inhibiting effect upon the cholera spirillum, the degree of inhibition apparently depending upon the thoroughness with which the acid albumins have been removed. Further, this inhibiting effect is more marked at body than at room temperature.

I have not been able to reproduce this phenomenon at every trial. Whether this is due to a variation in the amount of acid albumins removed on neutralization (it is well known that a portion of the precipitate formed redissolves in an excess of alkali), or to some undetermined cause, the fact remains that such a fluid will sometimes completely inhibit the growth of the cholera spirillum, but not that of B. coli. Work with chemically pure solutions might furnish another biological proof of the existence of ionproteid compounds and also an instance of *a specific* (?) *antitoxic action exerted by a proteid.* An instance of such an antitoxic action was shown by Kahlenberg and True[1] in relation to the seedlings of *Lupinus albus.* Hydrogen and various metallic kations, especially copper, were found to be toxic to the germinating seedlings, but the copper ion in combination with an organic complex had relatively little effect. In my case, however, the action may be due to free hydrogen ions from the HCl, since it is a well known fact that even small traces of acetic, hydrochloric, or citric acids completely prevent the growth of Spir. cholerae. Asiaticae.

The American committee does not recommend the removal of the acid albumins preliminary to the addition of the acid, but since Schultze-Schultzenstein[2] has shown that in fluids containing albumin or peptone or both, 0.097–0.217 per cent of HCl will destroy the cholera spirillum in an hour; and since further, as demonstrated by Smith,[3] hydrochloric acid is destructive to diptheria toxin; and again, since Ritchie[4] has pointed out a similar destructive action upon tetanus toxin, it was deemed advisable to leave the hydrogen ion out of the media.

The usual method of sterilization was followed with the exception that broth and agar were sterilized in the autoclave at 120° for half an hour. This prolonged sterilization at a high temperature was found necessary and expedient on account of

[1] JONES, H. C., *The Theory of Electrolytic Dissociation*, 1900, pp. 268-70.

[2] *Centralbl. f. Bakt.*, Abt. 1, 1901, 30, pp. 785-90.

[3] *Jour. of Exper. Med.*, 1899, 4, p. 383.

[4] *Jour. of Hyg.*, 1901, 1, p. 130.

some very resistant spored organisms encountered during the last hot season. It does not noticeably affect the nutritive qualities of agar or broth, although the initial acidity is raised about 0.5 per cent. When —1 agar or broth is subjected to such an autoclaving a precipitate is usually thrown down. On filtration and further autoclaving the alkalinity is found to have been diminished by about 0.5 per cent.

This discrepancy, according to the "Procedures," "is perhaps due to side reactions which are not understood." The usual formation of a precipitate, which seems to vary in proportion to the amount of alkali added, with the simultaneous reduction in the alkalinity of the medium, seems to point to the hydrolysis and separation of insoluble compounds under the influence of the alkali.

Contrary to a statement made in the footnote on p. 72 of the "Procedures," I have several times noted the evolution of ammonia when broth is boiled after the addition of the fixed alkali. As might be expected, this is most marked in the case of fermented broth, as illustrated in the following instance:

Three thousand c.c. of sugar-free broth had an initial reaction of +3.0; 2.5 per cent (75 c.c.) normal sodium hydroxide was added and thoroughly mixed. This should have given a calculated reaction of +0.5. The solution, containing a dense flocculent precipitate, was boiled for two minutes. During ebullition an appreciable quantity of ammonia was given off which could be detected both by the odor and by the blueing of red litmus paper moistened with distilled water. The solution was filtered, brought up to 3000 c.c. by the addition of distilled water, and then showed a reaction of +1.0—*i. e.*, the acidity had been raised 0.5 per cent over the calculated one.

A similar change occurs in unfermented meat extract, but here the escape of volatile alkali takes place more slowly.

THE SOURCE, ISOLATION, BIOCHEMICAL PECULIARITIES, AND VARIATIONS OF CULTURE "579."

NECROPSY NO. 579.

Emeterio Darita, age 28 years, male, Filipino, residence, Lorcha *Horatio* (boat on the Pasig River). Died April 17, 1903, after an illness of ten hours. Clinical diagnosis, cholera. Autopsy performed three and three-quarter hours after death.

SUMMARY OF POST-MORTEM FINDINGS.[1]

Skin of palms and soles shrivelled; fingers and feet in extreme flexion; thoracic and abdominal organs covered by a scanty adhesive secretion; lungs somewhat emphysematous: spleen small and rather soft; liver and kidneys normal in size, on section lobular, cortical and medullary markings

[1] For complete description, *cf.* forthcoming *Bulletin of the Bureau of Government Laboratories*, Manila, P. I.

indistinct; the intestinal tract showed a quantity of whitish mucoid substance: the lower third of the ileum was congested with patches of epithelial desquamation; the mesenteric glands and the solitary glands of the colon were enlarged.

ANATOMICAL DIAGNOSIS.

Cholera; acute follicular and necrotic enteritis; follicular colitis; acute parenchymatous nephritis; acute parenchymatous hepatitis; adhesive pleuritis; emphysema of the lungs; persistent thymus.

BACTERIOLOGICAL FINDINGS.

Tissues from the organs hardened in Zenker's solution. Ileum (carbol fuchsin 1:10): showed almost pure culture of slender rods, often curved, quite numerous; many degenerated, columnar epithelial cells. Spleen (Gram and safranin): no bacteria. Heart's blood (Gram and safranin): no bacteria.

METHOD OF ISOLATION.

Cultures were made from the ileum according to the Schottelius enriching method. Peptone solutions (one per cent Witte's peptone and 0.5 per cent NaCl in distilled water (with a reaction of +1, +0.5, neutral, and −1 were inoculated and kept at 35-37°.

In twenty hours the +1 tube showed a dense layer of growth near the surface, with beginning pellicle formation. A hanging drop preparation from the surface showed short, actively motile curved rods, apparently in pure culture.

The +0.5 and neutral tubes were well clouded, no pellicles.

The −1 tube was faintly clouded with a thin pellicle in the process of formation. It showed actively motile curved rods in the hanging drop.

The addition of ten drops of chemically pure sulphuric acid to each of the tubes gave a distinct indol reaction, which was most marked in the +1 tube.

Twenty per cent. gelatin plates inoculated from the surface growth of the +1 peptone tube were kept at 18-28°. In 20 hours pin-head sized areas of liquefaction were produced. These were circular, well defined and contained motile masses of growth of a broken up, refractile character.

A pure culture was obtained on +1 agar.

BIOCHEMICAL PECULIARITIES AND VARIATIONS.

On +1 agar a luxuriant dirty-white growth is seen in twenty-four hours at 35-37°. Later the edges become crenated—especially if the agar is somewhat dry. The condensation water is densely clouded. In old cultures spine-like processes may project from the edges of the growth, which is much more luxuriant on fresh moist agar than on the same medium with a slightly dry surface. (For the optimum reaction, see under "Liquefaction of Gelatin.")

In +1 broth the growth is somewhat variable. At one time the broth may be uniformly clouded (in the following, this is termed the "anaërobic type of growth"). Again it may be clouded with a dense layer of growth near the surface, which soon forms a pellicle (this is termed the "aërobic type of

growth"). In a stained preparation from a 48-hour culture the organisms are thicker and more curved than in one from peptone solution grown under like conditions. The morphology varies with the reaction of the medium. The character of the growth is independent of the presence or absence of muscle sugar, and is apparently due to the predominance of the aërobic type of the organism on the one hand, or of the anaërobic type on the other. I came to term these aërobic and anaërobic types of growth as a matter of convenience, but a better theoretical explanation is furnished by assuming that the difference is due to a variation in the specific gravity of the bacterial cells. If a number of broth tubes be inoculated from an agar slant, and kept under like conditions, some will show the aërobic type, while others will be uniformly clouded and may remain so or form a pellicle at a later date. The type of growth can be transmitted by further inoculations in broth, although, in the case of the anaërobic type, there is a tendency towards a cropping out of the aërobic one.

The production of a pellicle in fluid cultures.—As with the diphtheria bacillus, and other organisms, the habit of producing a pellicle in fluid cultures can be firmly established by transferring a portion thereof through a series of fluid cultures. "579 A" is one, which after being transplanted in this manner at intervals of three or four days for a couple of months, shows little or no tendency to grow in the deeper parts of the broth, while a dense layer appears near the surface of the fluid and a pellicle is formed in much less time than when the training process was initiated, and the same result can be obtained much more rapidly by using the pellicle formed on a liquefied gelatin culture. The whole process is, in fact, one of artificial selection. Inoculations from such cultures are usually made from the upper layers of the fluid and hence a series of such inoculations yields an artificially selected race of organisms of low specific gravity.

So far as indol and alkali production are concerned, there is no difference in the action of the aërobic and anaërobic type of organisms. (See alkali production.)

It is evident that the presence or absence of a pellicle in broth cultures is of little value in the differentiation of species.

Litmus milk is acidified and coagulated in 48 hours. (Control tubes remain sterile.) In about four days a firm clot is formed with separation of the whey and partial reduction of the litmus. The fermentation of lactose broth is evidence that this culture produces lactase.

+ 1 glucose broth is faintly clouded, but the growth occurs mostly at the bottom of the test tube as a stringy viscous mass. There is no apparent increase after 24 hours. (See growth in the fermentation tube.)

On potato (unneutralized) the growth is variable, sometimes none appearing, or again a slight dirty yellowish one may be seen in three or four days. This variability is probably due to a difference in the acidity of the potatoes used.

Solidified ox serum is rapidly digested.

When grown anaërobically (pyrogallic acid method), in *+ 1 glucose agar*, growth appears along the line of inoculation, but the culture is no longer viable after the second anaërobic transplanting.

a) *The cholera-red reaction.*— Immediately after isolation from the body, this organism gave a pronounced cholera-red reaction upon the addition of ten drops of chemically pure sulphuric acid to cultures grown in peptone solution during 18 to 20 hours at 35–37°. Since then this reaction has only appeared at intervals—even in solutions prepared from Witte's peptone,[1] which had been set aside as "proper for indol." All the cultures mentioned in this article have shown the same variation from time to time.

For some time, peptone solutions of various reactions were used both in isolating the cholera spirillum at autopsy, from stools, water, etc., and in testing for cholera-red, but without any constant results which might determine whether any one reaction favors surface growth or the demonstration of the reaction. Dunham's peptone solution containing one per cent Witte's peptone and 0.5 per cent sodium chloride in distilled water has a final reaction of +0.5, and has given the best results on the whole. This solution has such poor nutritive qualities for many species of intestinal bacteria that it is especially suitable for isolation by the Schottelius enriching method.

Upon investigating this uncertainty of the cholera-red reaction, I determined to try sugar-free broth, which has been shown by Smith[2] to be such an excellent culture fluid for the production of indol by bacteria. In the first batch of this medium these cultures gave excellent cholera-red reactions, but in two subsequent ones the reaction failed to appear. These three media were shown to be free from nitrites and fermentable sugars, by testing with B. coli, as recommended by Smith. In addition to sugar-free broth, four different peptone solutions were tested, namely: Peptone Sicca *cum* Sale, R. Nishiyama, Osaka, Japan; Peptone *e* Carne, E. Merck, Darmstadt; and two samples of Peptonum Siccum, Friedr. Witte, Rostock—one of which had been marked "proper for indol." These, too, were found to be free from fermentable

[1] It may be noted that this so-called "peptone" consists of a mixture of albumoses and contains only a minimal quantity of true peptone (TORALD SOLLMAN, *Amer. Jour. of Physiol.*, 1902, 7, p. 203); on the other hand, there are "peptones" on the market, such as that manufactured by the firm of Chapoteau, which contain as much as 50 per cent of pure peptone (J. P. PAWLOW, *The Work of the Digestive Glands*, 1902, p. 96).

[2] *Jour. of Exper. Med.*, 1897, 2. pp. 543–47.

sugars and nitrites, but all failed to yield cholera-red. However, they gave the indol reaction upon the addition of a trace of sodium nitrite.

As is well known, the demonstration of the cholera-red reaction depends upon the fact that an organism not only forms indol, but also reduces nitrates to nitrites. It seemed that the inconstancy of the reaction might depend upon a variation in the amount of nitrates present in different lots of peptone or meat extracts, or upon their accidental introduction on one occasion and not on another (when Cross and Blackwell's table salt is used, much more constant results are obtained, than when pure sodium chloride is employed). I prepared therefore peptone solutions in the manner above indicated, but in addition to the sodium chloride, I introduced 0.01 per cent sodium nitrate (one c.c. of a 10 per cent solution per liter). In such a solution the cholera-red reaction is not only constant, but it appears more promptly and is more intense than usual. Control peptone solutions, not containing sodium nitrate, failed to give the reaction. All the cholera cultures mentioned in this article give the reaction constantly and promptly in this medium. It seems probable that the use of Smith's sugar-free broth, containing 0.01 per cent sodium nitrate, would furnish a means of testing the production of indol and the simultaneous reduction of nitrates by many bacteria.[1]

b) *The liquefaction of gelatin.*—At the time of isolation, this organism showed active proteolytic properties—liquefaction of 20 per cent gelatin appearing within 24 hours, at 18–28°, and rapidly spreading to the sides of the test-tube as a shallow, circular, pan-shaped area. The liquefaction descended progressively, involving the whole width of the tube, with a slight funnel-shaped depression in the center along the needle puncture. Careful data concerning the reaction and dryness of the gelatin were not kept at the time, but some variations in the rate and character of liquefaction were noticed. Further work at the time being impossible, the original agar culture was kept in the ice-chest (transplants on + 1 agar being made at intervals of every two

[1] Since completing this work, I have found that as long ago as 1893, MAX BLEISCH (*Ztschr. f. Hyg., und Infektionskr.*, 1893, 14, pp. 103-115), emphasized the necessity of introducing nitrates into the peptone solution.

months) for eight months. The organism still showed the above type of liquefaction (often described as being characteristic of Sp. Finkler Prior) at 18–28° in 20 per cent gelatin, which had a final reaction of + 1.2. At the same temperature, in fresh 20 per cent gelatin, which had a final reaction of + 2, the organism slowly produced, in the course of three days, a small turnip-shaped area of liquefaction which, drying at the surface, left a small bubble-like depression—that is, it produced the type of liquefaction which was described by Koch as being characteristic of the cholera spirillum.

In two separate trials with the same gelatin (+ 2) at 35–37°, the inoculated material precipitated, and no growth or liquefaction occurred.

In +1.5 gelatin at 10–15° no growth occurred in 10 days, but rapid liquefaction took place on change to 18–25°.

When grown anaërobically (pyrogallic acid method) at 18–28° in +1 gelatin containing muscle sugar, growth appears along the stab, but no liquefaction takes place in three days.

Before detailing some experiments performed to determine the factors influencing variation in the type of liquefaction, it may be well to note some of the points brought out in the literature on this subject.

The proteolytic ferments of bacteria are only active in a medium alkaline to litmus, and it takes but a small amount of acid to hinder their action. This is in accord with the behavior of trypsin. When carbohydrates which can be so fermented as to form acids are present in gelatin, its liquefaction is inhibited. In 1898 Auerbach,[1] working with a number of liquefying bacteria, showed that the inhibiting power of glucose exceeded that of lactose; and that in the case of B. vulgare the acid products of fermentation inhibited the formation of the ferment itself. It seems that for the production of the ferment a medium containing albumin and the access of free oxygen are necessary. According to Liborius,[2] liquefaction of gelatin takes place very slowly in the absence of oxygen — with the exception of the case of some anaërobes.

According to T. Sollman,[3] "Kühne investigated the action of B. subtilis and B. prodigiosus, on solutions of protalbumose, from the chemical standpoint, and found that the phenomena resemble closely those of tryptic digestion. The conversion to tyrosin, leucin, and tryptophan was often almost complete." Again, according to Gotschlich,[4] "Kalischer, in experiments to

[1] *Centrabl. f. Bakt.*, 1898, Abt. 24, pp. 492–94.

[2] *Ztschr. f. Hyg.*, 1886, 1, pp. 115–76. [3] *Loc. cit.*, p. 211.

[4] *Handbuch der Pathogenen Mikroorganismen*, Kolle u. Wasserman, 1903, 1, p. 107.

determine how much of the casein splitting was due to the ferment and how much to the living cells, found that the ferment was able to produce peptone, leucin, tyrosin, as well as ammonia and aromatic oxyacids—in which its action also is in harmony with that of trypsin."

The melting point of gelatin undoubtedly plays a part in influencing the type of liquefaction which will occur at any given temperature. In my own experience the addition of alkali lowers the melting point. Thus, neutral gelatin which will not congeal at 18–28° will do so in the ice-chest, and +1 gelatin is not as solid as +1.5 gelatin at the same temperature. An interesting communication by Paul von Schroeder[1] throws some light on this subject: "When a gelatin solution is heated at 100°, and samples are taken out at intervals and placed in a thermostat at 25°, their viscosity being determined five minutes later, it is found that the values of the viscosity diminish, as the duration of the heating at 100° increases, ultimately becoming constant.

"This change is attributed to a process of hydrolysis. . . . .

"Certain salts increase the viscosity, magnesium salts exerting the greatest influence. . . . .

"The effect of hydrochloric acid and sodium hydroxide on the behavior of gelatin solutions was similarly studied. The process of hydrolysis is accelerated by both hydrogen and hydroxyl ions, and the final value of the viscosity thus attained after hydrolysis is lower than that reached in pure or salt containing gelatin solutions."

Again, according to Rousseau,[2] if gelatin be dialyzed, so as to remove the calcium salts contained therein, one obtains a solution which, sterilized in an autoclave at 120° for 20–30 minutes, solidifies upon cooling.

In order to determine what influence the reaction and dryness of the gelatin exert upon the type of liquefaction produced by a given cholera culture, the following experiment was performed: Nutrient gelatin was prepared containing 20 per cent gold label gelatin, one per cent Witte's peptone, and 0.3 per cent Liebig's beef extract. It was divided into halves and to each portion normal NaOH was added, one half receiving more than the other. After sterilization one portion showed a final reaction of +0.8, while that of the other was +1.0. In addition to this, another sample of gelatin, slightly darker in color, but prepared in the same way, which had been kept on ice for three weeks and showed some evaporation and a final reaction of +1.5, was used. This one was melted and resolidified before inoculation. Each sample contained a small amount of muscle sugar, as shown by subsequent fermentation with B. coli.

Four tubes from each of these samples were then inoculated from a 24-hour culture of "579" on +1 agar, which had been

[1] *Jour. of the Chem. Soc.*, 1903, 84, ii, p. 721. [2] *Bull. de l'Inst. Past.*, 1903, 1, p. 719.

kept on agar transplants at 35–37° for two and a half months. In 48 hours at 18–28° there was quite a noticeable variation in the amount of liquefaction produced in the different sets of tubes. The amount of this, in the four tubes of any one of the three sets, was not exactly uniform, probably on account of a variation in the number of bacteria introduced at the time of inoculation, but the difference between the three sets was very noticeable. Any one of the four +0.8 tubes showed more advanced liquefaction than any of the +1.0 tubes, and a like difference between the +1.0 and +1.5 tubes was still more marked.

It is often stated that "bacterial proteolytic enzymes, like trypsin, show increased activity in the presence of certain chemicals such as sodium carbonate and salicylate."

So far as the action of certain ions upon the tryptic digestion of fibrin is concerned, A. Kanitz[1] in reviewing the work of Dietz and confirming the quantitative experiments of Schields,[2] has shown that the optimum concentration of the hydroxyls from barium, strontium, and calcium hydroxides varies between $\frac{1}{70}$ and $\frac{1}{150}$ of the gram-molecule per liter. Determination of the electric conductivity and other physical constants shows that these alkaline earths are strongly and almost equally dissociated at these dilutions, and since the three hydroxides work at the same concentration he concludes that the kation is without influence and that the anion is alone active. He then calculated from the per cent of hydrolysis of potassium carbonate in given dilutions, the concentration at which the carbonate of potassium exerted the most active influence on tryptic digestion, and found this to be about $\frac{1}{200}$ of the gram-molecule per liter. He was unable to say that there was any difference in the mode of action of carbonate of potassium and the hydroxides of the alkaline earths. Kanitz concludes that the optimum for tryptic digestion is a liquid containing $\frac{1}{70}$ to $\frac{1}{200}$ of the hydroxyl ion (OH = 17 gms.) per liter.

In order to test the above statement from a bacteriological standpoint, an experiment was performed as follows: I prepared one liter of nutrient gelatin by adding 20 per cent Gold Label

[1] *Ztschr. f. physiol. Chemie.*, 1902, 37, pp. 75–80.

[2] *Ztschr. f. physik. Chemie.*, 1893, 12, p. 167.

gelatin, one per cent Witte's peptone, and 0.5 per cent sodium chloride to 1,000 c.c. of distilled water; the ingredients were then dissolved by boiling, distilled water added to 1,000 c.c., and the mixture divided into two parts of exactly 500 c.c. each; each half was titrated to phenolphthalein, and sufficient normal NaOH added to one half to give a reaction of +1, and an equal volume of normal Na_2Co_3 was then added to the other half; after cooling to 40°, the whites of three eggs were added to each half; each portion was then boiled for three minutes, filtered through cotton, distributed and sterilized in the Arnold for 20 minutes on each of three successive days. The final reaction of the NaOH gelatin was +1.7, while that of the Na_2Co_3 gelatin was +1.8.

Six tubes of each were then inoculated from the same place on the edge of the growth of a 24-hour agar culture of "579," and kept at 18–28°. Liquefaction commenced and progressed slowly but equally during several days' observation.[1]

An attempt was made to increase the proteolytic activity of this culture by transferring it at intervals of every few days from one gelatin tube to another. At the end of four months this culture showed no greater activity than another transplant of the same culture which had been kept on agar slants for the same length of time.

What has already been said, concerning the influence of the reaction of the medium upon the rapidity with which the cholera spirillum is able to liquefy gelatin, has a direct bearing upon the type of liquefied areas it will produce in or upon gelatin plates. Further, when, as has been noted by many observers, the same culture gives rise to two distinct types of liquefied areas, the difference may be explained by the relation of the plated organisms to their oxygen supply. Thus, an organism situated at the

[1] Note by Dr. Paul C. Freer, superintendent of Government Laboratories, P. I.: "The results of Kanitz would not appear to give conclusive proof of his view that the kation exerts no influence on the optimum reaction, as a glance at the table given in his paper will show, the variations between the three alkaline earths being quite marked at different temperatures. Dr. Wherry in his work compared equivalent amounts of sodium hydroxyde and sodium carbonate, and thus, while he had the same concentration of hydroxyl ions, he had, in the case of the latter reagent, 25 times the number of sodions present in unit volume. This latter fact would tend to show that the kation is without marked influence, at least in the case of sodion. However, these results show that the question is one which is barely touched and is well worthy of complete investigation by a use of the methods of physical chemistry in biology."

surface on account of its greater supply of free oxygen might be expected to produce a more rapidly spreading area of liquefaction than one more deeply situated, where the supply is *relatively* less. Moreover, the colony at the surface would be of the shallow turbid type with a greater area than that of the deeper colony where the organisms encounter a greater resistance of the surrounding gelatin, and in consequence of which they would be massed together—producing the refractile "ground-glass" type of colony. It has been noted that such "ground-glass" colonies, upon further growth, invariably break up into liquid areas with turbid contents, and such breaking up occurs, *pari passu*, with a lessening of the surrounding resistance and an increase in the supply of free oxygen. That such a supply of free oxygen does exist in a thin layer of gelatin can be proven by covering the opening of a gelatin stab culture with a few drops of liquid gelatin. Here no liquefaction occurs until the organisms have spread nearly to the surface.

c) *The optimum reaction.*—All cultures mentioned in this article show much more luxuriant growth in 18–20 hours, at 35–37°, on fresh —0.5 than on fresh +0.5 agar. Furthermore, the maximum amount of growth is obtained on —0.5 agar in 18–20 hours, while that on +0.5 agar does not equal it in 36–48 hours at the same temperature. The —0.5 agar was prepared from Liebig's beef extract, and had an initial acidity of one per cent acid to phenolphthalein. It was neutralized and brought to a reaction of —1. After sterilization, the reaction was reduced to —0.5. Since 20 c.c. of normal NaOH were added in the first place, and part of this was lost in the precipitate thrown down by autoclaving, it contained between $\frac{1}{30}$ and $\frac{1}{100}$ of a gram-molecule per liter. This would seem to support the idea that the optimum conditions for the growth and multiplication of the cholera spirillum are such as will favor its proteolytic activity.

d) *The production of alkali.*—Fifty c.c. of Smith's sugar-free broth[1] was placed in each of five 600 c.c. Erlenmeyer flasks and autoclaved at 120°. Final reaction, +1.5.

[1] For the method of preparation see *Jour. of Exper. Med.*, 1899, 4, p. 375.

Each was then inoculated with one loop from an 18-hour evenly clouded sugar-free broth culture of "579," and the following table illustrates the rate of alkali production:

TABLE I.

No. of Flask	Temperature	Age of Culture	Reaction to Phenolphthalein	Remarks
1.....	30°–35°	24 hrs.	+0.8	Dense cloudiness; no pellicle
2.....	30°–35°	48 hrs.	+1.0	Dense cloudiness; no pellicle; actively motile curved rods
3.....	30°–35°	72 hrs.	+0.2	Do.
4.....	30°–35°	96 hrs.	Neutral	Do.
5.....	30°–35°	120 hrs.	−0.8	Dense cloudiness; no pellicle; rods not so actively motile; few curved filaments

Alkali production progresses equally well when a pellicle is formed. It also occurred in unneutralized sugar-free broth with an initial acidity of +2.3. If the sodium chloride usually added to sugar-free broth be left out, no formation of alkali can be detected by titration with phenolphthalein—at least during five days. In control flasks of the same broth, plus sodium chloride, alkali production occurred about as rapidly as shown in the above table. This would seem to indicate that the alkali is produced by a conversion of a NaCl into NaOH or Na_2Co_3, and that the greater part of the alkalinity is owing to the formation of such substances rather than to ammonia, amine, and ammonium bases, to which it is usually attributed. However, it is also possible that sodium chloride exerts a catalytic (accelerating) action on the formation of ammonia.

DESCRIPTION OF THE SOURCE AND ISOLATION OF FIVE OTHER CHOLERA CULTURES,[1] AND OF THEIR RESEMBLANCE TO ONE ANOTHER AND TO CULTURE "579."

Cholera "Scout" is a culture obtained by the Schottelius enriching method from a stool sent to the laboratory from Caloocan on April 16, 1903. The patient was a native scout who died the next day with typical symptoms of Asiatic cholera, which was epidemic in Caloocan and the surrounding country at the time. In its cultural characteristics it is indistinguishable

[1] During this comparative study every precaution was taken to avoid contaminating one strain with portions from another culture, and the purity of each was controlled by frequent microscopical examinations and by plating in gelatin or agar.

from "579," excepting that litmus milk is acidified in 24 hours at 37°, but no coagulation occurs in five days. Hence, like "579" it produces lactase, and differs from it in the absence of the production of rennin.

Cholera "561" is a culture obtained by the Schottelius enriching method from Eugenia Holandes, a Filipina, 33 years old, who died on March 26, 1903, after an illness of three days. Autopsy seven hours after death. To summarize briefly the postmortem findings: The skin of the hands and feet are shriveled; the feet are in extreme flexion with the toes in extension; the pleural and peritoneal membranes covered with a sticky secretion. Cloudy swelling of the solid organs. Ileum congested, especially in its lower half, the mucosa showing some patches of epithelial desquamation. Contents of ileum greenish black and containing much mucus. Old and advanced amœbic colitis. Culturally it is indistinguishable from Cholera "Scout."

Cholera "A" is a culture obtained at autopsy by Dr. Strong some time in the fall of 1903, cholera being endemic in Manila at the time. Culturally it is indistinguishable from Cholera "Scout."

Cholera "City Moat" is a culture obtained by Mr. Lindquist, of the First Reserve Hospital Laboratory, from the city moat near the hospital about July, 1903. Cholera was endemic in Manila at the time. Culturally it is indistinguishable from Cholera "Scout."

Cholera "Pfeiffer" is a culture of that name brought by Dr. R. P. Strong from Germany. It has been grown on artificial media for a period of nine years, and during the past year has not been passed through animals. Culturally it is indistinguishable from Cholera "Scout," but it is very much more sensitive to the action of agglutinating sera.

Cholera "554-B" is a culture obtained on March 20, 1903, from a cholera autopsy. Morphologically it appears as short, curved, actively motile rods. It closely resembles the above cultures, but does not agglutinate with the serum of a rabbit immunized against "579," nor with the serum of a cholera convalescent.

GROWTH IN THE FERMENTATION TUBE IN THE PRESENCE OF CARBOHYDRATES.

Medium: Smith's sugar-free broth, which had a final reaction of +1.5 containing one per cent of glucose, maltose, saccharose, and lactose. One per cent of starch was added to some of the same broth and autoclaved after distribution; the initial reaction was not changed. It was inoculated with "579" and kept at 35–37°.

In glucose, maltose, and saccharose broth there was growth in the closed arm as well as in the bulb, and the acids produced were of such a character as to destroy the vitality of the organism. On the other hand, in the case of the lactose and starch broth, no growth occurred in the closed arm, and although a greater quantity of acid was produced, the organism was still viable on the fourth day. The following table gives the results noted:

TABLE II.

Sugar-free Broth with One Per Cent.	Gas	Reaction of Contents of Bulb and Neck on Fourth Day	Gas in Control Tubes Inoculated with B. coli Fourth Day	Remarks
Glucose	0	+3.3	30%; $\frac{H}{CO_2} = \frac{2.5}{1}$	Maximum growth attained in 24 hours. Bulb and closed branch turbid; no pellicle. Agar slants inoculated after 24 hours from the bulb or neck remain sterile.
Maltose.....	0	+3.2	45%; $\frac{H}{CO_2} = \frac{3}{1}$	Do.
Saccharose..	0	+3.5	No gas; closed arm cloudy	Agar slants inoculated on the fourth day from the bulb or neck remain sterile. Do.
Lactose.....	0	+4.0	40%; $\frac{H}{CO_2} = \frac{2}{1}$	Maximum growth attained in 24 hours. Bulb and neck turbid, *closed branch clear;* no pellicle. Growth more dense than in other sugars. Agar slants inoculated on the fourth day from bulb or neck show a luxuriant growth in 24 hours at 37°; actively motile curved rods in the hanging drop.
Starch[1].....	0	+4.0	No gas; closed arm cloudy	Maximum growth attained after 24 hours. Culture viable on fourth day, as per lactose tube.

In another series of experiments, in which 0.5–1.0 per cent glucose broth (final reaction = +1.5) was distributed in small flasks and inoculated from the same culture and kept at 35–37°, the maximum amount of acid (3–3.5 per cent) was produced in 24 hours, and transplants made at that time remained sterile.[2]

Again, enough normal NaOH was added to sugar-free broth to give a calculated neutral reaction. The final reaction after auto-

[1] A test tube containing the same starch solution became densely turbid, and a well-marked pellicle was formed. On the fourth day the acidity had reached three per cent. The closed arm of a fermentation tube was filled with this culture and —1 broth added. When inoculated with B. coli 30 per cent of gas was formed in 48 hours $\frac{H}{CO_2} = \frac{3}{1}$.

[2] See analogy in the case of the diphtheria bacillus (TH. SMITH, *loc. cit.*, p. 382). It is extremely probable that any toxin formed by the cholera spirillum would be destroyed in a manner similar to that which takes place in diphtheria cultures.

claving was +0.7. A sterile solution of glucose, amounting to $\frac{1}{4}\frac{1}{0}$ per cent was then added and the flask inoculated and kept at at 28°. In four days the acidity had been raised 0.5 per cent, and the culture was still viable. The experiment was not carried on for a sufficient length of time to note whether the acid produced would be finally neutralized by such alkali production as normally takes place in sugar-free broth, but this is hardly probable, as the growth, in the presence of even such a small per cent of glucose, is rapidly precipitated, and forms a very viscous sediment.

The other cultures grown in solutions of these carbohydrates (reaction +1–+1.5) yielded similar results as shown in the following table:

TABLE III.

Broth	"Scout"	"561"	"A"	"City Moat"	"Pfeiffer"	Remarks
Glucose	+3.8	+3.6	+3.8	+3.5	+3.5	Titration on 4th day. Character of growth and fate of culture as per culture "579"
Maltose	+4.0	+4.0	+3.0	+4.0	+4.4	Do.
Saccharose .	+3.0	+3.0	+3.0	+3.0	+3.0	Do.
Lactose.....	+3.8	+4.0	+4.5	+4.3	+4.0	Do.
Starch	+2.8	+3.0	+3.0	+2.3	+2.8	Do.

Buxton,[1] in an excellent discussion on bacterial enzymes states that "Cholera then produces amylase, maltase, but no invertase, lactase nor inulase." These cultures seems to produce both lactase and invertase. The sugars used were prepared by Merck.

E. Gotschlich[2] states that Fermi and Montesano found that invertin occurred inconstantly in the cholera spirillum and spirillum of Metchnikoff.

RELATIONSHIP AS SHOWN BY AGGLUTINATING AND BACTERICIDAL SERA

In applying the Gruber-Durham test to the study of the identity or relationship of the following cultures, a number of facts

[1] *Amer. Med.*, July 25, 1903, 6, p. 138. [2] *Loc. cit.*, p. 103.

observed by others influenced both the choice of the method employed and the interpretation of the results.

On account of variations in the density of the growth in broth, which the cultures studied at times show, emulsions of the bacilli in 0.8 per cent sodium chloride solution were exclusively employed. The cultures were grown on +1 agar for 18–20 hours at 35–37° and the emulsions made to correspond as nearly as possible with the density of a 24-hour typhoid culture according to the method employed by Smith[1] in the comparative study of tubercle bacilli. They were allowed to stand for 10 minutes in order to give time for the coarser particles to settle. Such an emulsion is microscopically free from clumps and the rods retain their active motility in the control drops for an hour or more,

The serum was diluted with 0.8 per cent sodium chloride solution in Thoma-Zeiss blood pipettes, and a loopful of this serum was then mixed with an equal quantity of the emulsion and examined from time to time with the $\frac{1}{6}$ objective. It will be noted that the dilution of the serum in the drop was always twice that in the diluting pipette. Control hanging drops of the emulsion were always made and examined before and at the close of each experiment. The microscopic method was employed because it was believed that the end of the reaction can be more accurately determined and any differences in the character of the clumps noted.

It is a well known fact that organisms, which have been grown for a long time upon artificial media, are more sensitive to the action of homologous sera than they are when their pathogenicity has been raised. Typhoid cultures recently isolated from the body sometimes show a marked resistance to agglutination with the patient's serum as compared with old laboratory cultures. As shown by F. Hamburger[2] the agglutinability of cholera cultures diminishes with an increase in virulence.

AGGLUTINATION WITH THE SERUM OF A CHOLERA PATIENT.

The history of the serum is briefly as follows: Candido Nugin, a Filipino, 19 years old, died at the San Lazaro Cholera Hospital on January 8, 1904. He was ill for 13 days; had rice-water stools during the acute stage of the disease, passed into the typhoid stage, and died on the 13th day with

[1] *Jour. of Exper. Med.*, 1898, 3, p. 465. [2] *Wien klin. Wchnschr.*, 1903, 16, p. 97.

symptoms of acute nephritis. At the autopsy six hours after death, the kidneys showed acute parenchymatous nephritis; there was cloudy swelling of the liver and heart muscle. The ileum was still in a congested state, but its mucosa was in fairly good condition. Smears from the ileum showed a number of thin curved rods, mixed with many other organisms. No cultures were made.

The following table shows the agglutinating action of the serum from the heart's blood of this patient in such dilutions as were tested. An accident to the serum prevented the determination of its agglutinating limit.

TABLE IV.

Culture	Temperature	Dilution of Serum	Result
"579"	28°	$\frac{1}{100}$	Small motile clumps in 17 minutes; complete in 30 minutes
"579"	28°	$\frac{1}{200}$	Small motile clumps in 10 minutes; complete in 40 minutes
"Scout"	28°	$\frac{1}{100}$	Almost complete in 30 minutes; complete in 60 minutes
"561"	28°	$\frac{1}{100}$	Almost complete in 30 minutes; complete in 60 minutes
"A"	28°	$\frac{1}{100}$	Almost complete in 30 minutes; not complete in 60 minutes
"City Moat"	28°	$\frac{1}{100}$	Complete in 30 minutes
"Pfeiffer"	28°	$\frac{1}{100}$	Complete in 20 minutes
"554 b"	28°	$\frac{1}{40}$	Negative during an hour's observation

My own serum diluted 1:20 produced no agglutination during 45 minutes' observation.

In this experiment no attempt was made to use salt solution, suspensions of equal density and the variation in the time when complete agglutination occurred is noticeable.

AGGLUTINATION WITH IMMUNE RABBIT SERUM.

A rabbit was injected with 0.8 per cent sodium chloride suspensions of culture "579" grown on + 1 agar for 24 hours at 35–37°. It received the contents of about six agar slants subcutaneously and intraperitoneally during two months. In this 24-hour old serum the agglutinating limit is not great, but is considered sufficient for the following comparative and quantitative estimations:

TABLE V.

Culture	Temperature	Dilution of Serum	Result	Remarks
"579".....	25°	$\frac{1}{800}$	+	[1] Complete in 35 minutes; small compact clumps
"579".....	25°	$\frac{1}{2000}$	−	[1] Partial in 25 minutes; not complete in 60 minutes
"Scout".....	28°	$\frac{1}{800}$	+	[1] Complete in 35 minutes; small, loose clumps
"561".....	28°	$\frac{1}{800}$	+	[1] Complete in 35 minutes; small, loose clumps
"A".....	28°	$\frac{1}{800}$	+	[1] Complete in 35 minutes; small, compact clumps
"City Moat"..	28°	$\frac{1}{800}$	+	[1] Complete in 30 minutes
"Pfeiffer".....	28°	$\frac{1}{800}$	+	[1] Complete in 35 minutes; small, loose clumps
"554 b".....	28°	$\frac{1}{200}$	−	Negative in 35 minutes

The normal serum of a control rabbit gave no agglutination at 1:10 in 45 minutes at 28°.

All of these cultures have been grown upon artificial media for from six to twelve months, with the exception of "Pfeiffer," which, as already stated, has been grown on artificial media for the past nine years. Cholera "Pfeiffer" agglutinates almost immediately at a 1:40 dilution, whereas it takes several minutes to produce complete results with the other cultures at this dilution. This susceptibility is not noticeable at the higher dilutions.

PFEIFFER'S REACTION (PERFORMED IN VITRO AFTER THE METHOD OF BORDET).

A loopful of sodium chloride suspension of the culture to be tested was mixed with a loopful of the above mentioned immune rabbit serum and the result watched in the hanging drop. All of the cultures, with the exception of "554b," agglutinated, the rods became swollen and globular, and in about three hours, at 28°, began to break up into granular masses. "554b" agglutinated, the rods became swollen but did not disintegrate. In control drops of immune serum alone the rods agglutinated, but no bacteriolysis occurred. In control drops of normal serum the rods retained their motility for three hours.

[1] The hanging drop was not examined during the five minutes previous to the given time—hence, it is probable that the table indicates a greater uniformity in this respect than occurred in reality. (See footnote under Morphology and Pleomorphism.)

PATHOGENICITY.

GUINEA PIGS.

Only the pathogenicity of culture "579" toward these animals has been tested. At the time of isolation about two c.c. of a 24-hour broth culture injected intraperitoneally killed a fair-sized guinea pig within 24 hours. Eleven months later, after direct passage through three guinea pigs, one loop[1] of a 24-hour + 1 agar culture, grown at 35–37°, killed a 482-gram guinea pig in four hours. The peritoneal and thoracic cavities showed intense congestion with sero-sanguinous extravasations. The small intestine was greatly congested (much more so than the large) and filled with a yellowish mucoid fluid containing many desquamated epithelial cells, but, microscopically, no cholera spirilla. The abdominal organs were bound together by a fibrinous exudate. Pure cultures were obtained from the peritoneal cavity on agar plates. There were no organisms in the heart's blood.

PIGEONS (full grown and of about the same size).

Cultures "579," "Scout," "City Moat," and "561" were pathogenic when one loop of a 24-hour — 1 agar culture suspended in salt solution was injected deep into the pectoral muscle. One loop of culture "A" failed to kill a pigeon. Five loops (about 30 m.g.) of culture "Pfeiffer" failed to kill. Abstracts of the protocols are as follows:

Pigeon 1.—One loop of "579" deep in left pectoral muscle. Dead in 54 hours. Congestion of cutaneous and deep vessels of left side. Cloudy swelling of left pectoral. Intestines congested. Microscopically, many curved rods in left pectoral; none in heart's blood. Many halteridia and shadow corpuscles in blood. Pure cultures were obtained from the left pectoral muscle and heart's blood (three colonies per loop), which agglutinated with the "579" immune rabbit serum at 1:200 in about 20 minutes.

Pigeon 2.—One loop of "Scout" deep in left pectoral; dead in 20 hours; tissue changes as in Pigeon 1; organisms present in pectoral and heart's blood microscopically; many halteridia present; pure cultures from pectoral and heart's blood which agglutinated with "579" rabbit serum at 1:200.

Pigeon 3.—One loop of "City Moat" deep in left pectoral; dead in 34 hours; tissue changes similar to first case; organisms numerous at seat of

[1] The same loop was used throughout the following experiments. When it holds just sufficient culture to fill the cavity of the loop and form a rounded surface on each side, its contents weigh seven m.g. (wet). Allowing one m.g. for loss during manipulation, "one loop" signifies about six m.g. of the culture.

injection, not found microscopically in heart's blood, which contained numerous halteridia and many shadow corpuscles. Pure cultures obtained from pectoral and heart's blood, which agglutinated with "579" rabbit serum at 1 : 200.

Pigeon 4.—One loop of "561" deep in left pectoral; dead in 44 hours; tissue changes as above; curved rods at site of injection and quite a number in the heart's blood; few halteridia; cultures from pectoral and heart's blood pure, and agglutinate with "579" rabbit serum at 1 : 200.

Pigeon 5.—One loop of "A" deep in left pectoral; alive and well on tenth day; blood from foot shows very few halteridia.

Pigeon 6.—One loop of "Pfeiffer" deep in left pectoral; alive and well on tenth day. No halteridia found in blood from foot.

Pigeon 7.—Two loops of "Pfeiffer" deep in left pectoral; alive and well on sixth day. No halteridia found in blood from foot.

Pigeon 8.—Five loops of "Pfeiffer" deep in left pectoral; alive and well on fourth day. Blood from foot shows a number of halteridia.

The dose injected into these pigeons was rather large, but was adopted on account of the age of the cultures. I have not been able to test the relative resistance of a pigeon showing marked halteridium infection on the one hand and one free from it on the other hand, on account of the difficulty of obtaining uninfected pigeons. Culture "Pfeiffer" has been grown on artificial media for such a great length of time that it could hardly be expected to be pathogenic except in large doses. (See footnote under "Morphology and Pleomorphism.")

MONKEYS.

Several attempts to infect monkeys by feeding have been performed with negative results, but I have notes on one case only. An old adult male monkey (Macacus cynamolgus) received the contents of a recent agar slant culture of "579" suspended in —1 broth. This was injected by means of a catheter into the stomach. He remained perfectly well for 24 hours. During the next four days 35 c.c. of native spirits, called "arac" (containing about 40 per cent alcohol), were injected into his stomach. During part of the time he appeared to be intoxicated and refused to eat. Five c.c. of one per cent sodium carbonate was injected into his stomach, followed by the contents of three +1 agar slants of "579" suspended in —1 broth. He did not vomit; feces were normal, and he remained well during a week's observation. The culture "579" had been grown on artificial media for nine months without passage through an animal.

MORPHOLOGY AND PLEOMORPHISM.

Each of these cultures shows a tendency toward pleomorphism, which is quite as marked as that seen in cultures of B. pestis or B. mallei.

It is generally admitted that no two separate lots of media, which are identical in composition and reaction, can be prepared; and though the methods recommended by the American Committee, and the somewhat modified ones employed in these experiments, give comparable results so far as the reaction may indicate uniformity in composition, some bacteria will point out variations not detectable in other ways. I have not been able to reproduce exactly the same type of morphology in any two successive cultivations of the same culture, even on agar from the same batch, although precautions were taken to grow the cultures under apparently identical conditions, to make the preparations from corresponding portions of the growth, and to subject them, as nearly as could be judged, to like conditions of heating, staining, etc. On agar made on separate occasions the variation is still more marked.

In the case of the cholera spirillum, which reaches its maximum growth on moist agar in such a short time, variations in morphology will be shown in preparations from the same culture. Thus, one made from the *edge* of a streak on an agar slant, where the younger forms are still multiplying, may present an entirely different appearance from one made from the *center* of the streak, where the older forms have lengthened out and are undergoing involution changes.

The variation which is so striking in this instance is not always so apparent in any of the cultures under consideration, nor is it appreciable in a 24-hour culture of the less pleomorphic *B. coli*. Still this difference in the morphology of the younger and older forms of the cholera spirillum must be taken into consideration in comparing the morphology of different cultures, and when it is taken into account the variation on agar from the same batch may not be so marked.[1]

[1] It seems worthy of note that in the preparation of the saline emulsions for the agglutination and animal inoculation experiments heretofore cited no such precautions were taken. It seems that the failure to do so may account for some of the variations observed.

It is hardly necessary to say that no permanent variations in morphology were produced in any culture. In one culture which was kept in broth for 10 months, the organisms were somewhat larger than in other cultures. This is but a temporary modification, transmitted while the culture is kept in broth, but very soon reverting to the shorter, thinner type when grown in peptone solution or transplanted upon +1 agar.

The long straight and spiral threads, which grow out in the pellicle formed on a liquefied gelatin culture, are undoubtedly involution forms, for when a portion of such a pellicle is transplanted upon +1 agar, short curved forms such as are ordinarily met with upon agar develop abundantly. Further, in stained preparations from such an agar surface these threads and spirals take the stain poorly and after two or three transplants disappear entirely.

SUMMARY.

1. The substance of this article consists, essentially, in a careful preliminary study of the variations which occur in one culture of the cholera spirillum, and a comparison of this with cultures from different sources.

2. Certain reasons are given for adopting a modification of the methods of neutralizing media recommended by the American committee—the hydrogen ion being left out on account of its toxic action.

3. The cholera spirillum is not a nitrifying organism, and the successful demonstration of the "cholera-red reaction" in a solution of Witte's "peptone" depends upon the presence of a trace of nitrates. Certain reasons are given for presuming that a variation in the nitrate content of media exists.

4. The type of liquefaction produced in gelatin is influenced to a marked degree by the reaction and melting point of the gelatin. Sodium carbonate does not exert a more favorable influence on the proteolytic activity of the cholera spirillum than sodium hydroxide—at least so far as the liquefaction of gelatin

It is not at all impossible, for example, that a "loop" of young, healthy cholera spirilla, taken from the edge of the growth on an agar slant, would exert a greater pathogenic action than one taken from the center, where the growth is composed of old, semi-degenerate individuals.

is concerned. The proteolytic activity of a culture could not be increased by passage through a series of gelatin tubes.

5. The optimum condition for growth is furnished by an albuminous medium containing between $\frac{1}{50}$ and $\frac{1}{100}$ of a gram-molecule of NaOH or Na_2CO_3 per liter, and this corresponds fairly well with the optimum conditions for the tryptic digestion of fibrin.

6. Alkali, detectable by titration with phenolphthalein, is not produced in sugar-free broth devoid of sodium chloride.

7. Growth in the presence of carbohydrates reveals that the acids produced from glucose, maltose, and saccharose rapidly kill the cholera spirillum, while those produced from lactose and starch are not toxic—at least within a given time.

8. The cultures studied are specifically the same as shown by the Gruber-Durham and Pfeiffer reactions. In order to obtain comparable results, quantitative variations between the agglutinin and agglutinable substance were excluded as far as possible.

9. The pathogenicity for guinea pigs, pigeons, and monkeys is mentioned.

10. Upon comparing the morphology of the different cultures it was noted that if precautions be taken to make preparations from corresponding portions of the growths, the variations were not so marked.

In conclusion, I wish to express my gratitude to Dr. Paul C. Freer, Superintendent of Government Laboratories, for many valuable corrections of the chemical concepts put forth in this paper, and for helpful suggestions in its editing.

AMEBAS:* THEIR CULTIVATION AND ETIOLOGICAL SIGNIFICANCE.†

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INTRODUCTION.

The cultivation of amebas,‡ while a subject of the greatest importance, has never excited a very general interest, especially in America; and, although there is an extended literature on the subject, it is scattered over a long period of time and unfortunately some of the earlier writings deal with work not performed with proper care.

This early and unsatisfactory work accounts partly for the small amount of attention which the more careful articles of recent times have received, and materially adds to the responsibility of those who now take up the subject. Some of the confusion and doubt which exist in regard to cultivation and etiology is also due to the fact that a number of those who have grown amebas have, although insufficient data were at hand, devoted much time to a discussion of the biology and classification of the parasites.

The controversy relative to the etiological significance of these parasites in human disease has been active almost since the time of the very important observations of Lösch, and, to judge from much of the recent literature, is still far from being decided. Any attempts at the solution of these problems must take this doubt into consideration, and every detail of the subject must therefore be made clear.

In this paper we have purposely avoided a detailed discussion of the life cycle, the classification, and other strictly biological questions regarding amebas, but instead have taken up their cultivation from various sources, including the dysenteric intestine,

*The authors have followed Dorland's Dictionary in spelling.

†Received for publication Dec. 3, 1904.

‡A full and detailed account of our experiments together with the bibliography is given in Bulletin 18 (1904) of this laboratory.

and have proved their etiological rôle by animal experiment with cultures of diverse origin, and have shown that at least some amebas are pathogenic. Certain of these pathogenic ones are found in places which indicate methods for the prevention of the infection, a consideration which is of great importance to us in the Philippine Islands.

DISTRIBUTION.

Amebas are widely distributed in nature. They have been isolated from soil (both surface and deep), from marshes, thermal springs, rivers, lakes, sea water, air, dust, dried grass, fruits, vegetables, and many other substances. Amebas are also present in the intestinal canal of many animals. They have been reported in the frog, chicken, pigeon, lamb, calf, rabbit, dog, horse, and monkey. In human beings amebas have been found in the stomach, intestine, liver, bladder, vagina, urine, necrotic bone, tartar of the mouth, and in gangrenous wounds associated with liver abscesses.

CULITIVATION.

Desultory attempts at the cultivation of amebas have been going on for some years. In practically all of the successful ones the amebas have been grown in the presence of bacteria. We have repeated the work of the principal experimenters along this line, but usually with negative or unsatisfactory results, so far as the cultivation of amebas from the intestine is concerned.

A large variety of media, both fluid and solid, may be used for the cultivation of amebas from water, hay, soil, etc., but for the cultivation of amebas that have been passed through the alimentary canal of man and other animals the choice is not great. As a stock medium we have come to use the following, prepared in the same manner as ordinary agar:

Agar - - - -	20 grams
Sodium chloride -	.3 to .5 grams
Extract of beef - -	.3 to .5 grams

to the litre of finished product.

This is most satisfactory with one per cent normal alkalinity to phenolphthalein. As autoclaving the media increases the acidity it will usually be necessary to start with an initial alka-

linity of 1.5 per cent. Variations from this stock medium, consisting in the use of a still smaller amount of sodium chloride and beef extract, or in leaving out the salt entirely, will sometimes be found advantageous, especially when the amebas are growing in company with a luxuriant saprophytic bacterium. On the other hand, a small quantity of peptone sometimes improves the medium when a delicately growing bacterium is being used.

The isolation and cultivation of amebas from water and most external sources is usually quite easy. To do this, place 100 to 500 c.c. of the water, aqueous solution or suspension of the substance to be examined, in a sterile flask and to each 100 c.c. of this sample add one-half to one c.c. of broth of a one per cent normal alkalinity, and set aside for one to three days. The amebas, if present, collect at the surface of the fluid. Thence they may be transferred by a platinum loop to agar plates. Bacteria in sufficient number to ensure the growth of the amebas are usually transferred with them. In the course of six to 48 hours, by an examination with the low power of a microscope, amebas can be seen and transfers can be made.

In attempting to cultivate amebas from feces a preliminary examination to determine the presence or absence of these organisms is important. This examination is always facilitated by giving the patient a saline carthartic and examining the fluid portion of the stools. The carthartic flushes out the entire colon, increases the number of the parasites in the stool, and thus renders the examination easier and the results more reliable, The diagnosis should never be made upon non-motile forms. Resting and encysted forms may ordinarily be diagnosed with reasonable certainty, but mistakes are occasionally made.

We have never cultivated amebas from the stools by first inoculating them into fluid media of any kind. We have occasionally secured growths by lightly smearing the surface of agar plates with material selected from feces. In these cases other organisms were probably carried over in sufficient numbers to nourish the amebas. The percentage of successes may be increased if the surfaces of several plates are previously smeared with pure cultures of various bacteria known to favor the growth

of amebas. By using 12 different selected bacteria in this way we have succeeded in obtaining growth in 30 per cent of one series of cases, where control inoculation made on the same medium but without bacteria showed only two per cent of growths.

Where these means fail, growth may sometimes be secured by first plating out the bacteria from the stool that is being examined, and then inoculating them in pure culture upon a series of plates before smearing with the amebas. By this method we have succeeded in cultivating the amebas in about 60 per cent of the cases where all other methods had failed. At times we have cultivated amebas from stools in which they were not microscopically demonstrable.

The first plates from the stools or from intestinal ulcers must be watched frequently and carefully under the microscope, in order that transfers from them may be made as soon as the amebas develop. Unless these transfers are promptly made, the amebas die. In certain cases in which the amebas resisted cultivation growth has been obtained only after causing them to encyst. After the amebas have become accustomed to the artificial media there is usually less trouble in their growth.

Many attempts have been made to grow amebas in the absence of bacteria. In order to eliminate the bacteria, heat, filtration, negative geotropism, animal inoculation, and the use of disinfectants and special media have been tried. Few if any of these attempts have proved successful, and even in those cases in which the parasite was supposed to have been satisfactorily isolated, development did not take place when transplants were made.

Tsujitani[1] obtained amebas in pure cultures with cholera vibrios and then destroyed the latter by heat. The encysted amebas transplanted to sterile media became active but did not multiply until transferred to living or to killed cultures of bacteria.

The only environment in which amebas are found to be apparently free from bacteria is that furnished by certain culturally bacteria-free liver abscesses. If these abscesses in reality do not contain other organisms, it probably proves that

[1] *Centralbl. f. Bakt.*, 1898, Abth. 1, 24, p. 666.

there are substances other than those furnished by microorganisms that are capable of nourishing amebas under certain conditions. We advance the theory that these substances are probably enzymes. All attempts to obtain pure cultures by reproducing artificially a condition similar to that in the bacteria-free liver abcess have failed. We have employed many methods, including those of other workers, as well as a considerable variety which have not been described, but always with negative or doubtful results.

Satisfactory pure cultures of amebas have not yet been obtained, and the work of all recent authors as well as our own seems to point to the impossibility of such a procedure. A symbiotic microorganism seems indispensable for the nourishment of these protozoa.

To cultivate a single species of ameba, transplantation of a single individual is usually necessary. To do this an agar plate with well distributed parasites is placed, open side up, on the microscope stage. With the low power an ameba is selected that is distant one or more fields from any other, and a clean, perfectly dry, high power lens is swung in place. This is lowered until its entire surface is in contact with the medium and then raised quickly. If the ameba has been picked up, the lens is removed and gently rubbed on the surface of a second plate. One may sometimes obtain by this method a pure culture of amebas in symbiosis with a pure bacterial culture.

To cultivate a single species of ameba with a pure bacterial culture, the following routine has been developed in our work. It is based upon principles maintained by Beyerinck,[1] Mouton,[2] and others. By means of a platinum loop, several concentric rings, of a pure culture of the bacteria with which the amebas are to be grown, are drawn upon the surface of an agar plate, and a small smear of the amebas is inoculated in the middle of the inner ring. In from 24 to 72 hours the amebas will have passed outward across one or more of the rings. In crossing the rings they lose the organisms with which they started and take up those forming the

[1] *Centrabl. f. Bakt.*, 1895, Abth. 1, 19, p. 257.

[2] *Ann. de l'Inst. Pasteur*, 1902, 16, p. 457.

rings. It sometimes happens that they appear in pure culture with the desired organism at the circumference of the first plate, but generally one or more transfers to similarly prepared plates are necessary, the amebas being taken each time from outside the largest ring and inoculated within the smallest ring of the next plate.

By this method the symbiotic value of the bacteria forming the rings may be determined as the behavior of the amebas varies according to their liking or antipathy for the bacteria forming the rings. If the amebas are attracted by the bacteria, they cross the rings quickly. If there is a slight antipathy for the bacteria the amebas are delayed. If this antipathy is greater, they refuse to cross the ring, and if it is very great, they encyst upon coming up to it.

When it is desired to transfer amebas from a highly satisfactory symbiotic bacterium to one that is less satisfactory, it is often convenient first to grow the amebas upon a mixed culture of the two organisms, before isolating them with the desired bacterium. When the two organisms differ greatly in the profusion and rapidity of their growth, the nutriment in the media may be varied or the rings of the delicately growing organism may be allowed to grow 24 to 48 hours before inoculating the amebas in the centre of the plate.

Amebas show a selective action for certain bacteria. If plates are made from a substance in which amebas are multiplying, and the various bacteria are isolated in pure culture, it will be found that the amebas will multiply profusely with some, will grow indifferently well with others, while with certain ones no growth whatever will occur. This selectiveness varies with different amebas. After cultivating certain amebas for three months we attempted to grow them upon 18 stock cultures of bacteria. One ameba grew well on 16 and poorly on 2 cultures. A second grew well on 11, poorly on 6, and not at all on 1. While a third ameba grew well on only 1, poorly on 11, and not at all on 6.

Amebas from the human intestine and other parts of the animal body are particularly selective, and cultivation from these sources is difficult, and this difficulty tends to become progress-

ively greater the longer the amebas remain in the animal body. On the other hand, amebas from external sources, as a rule, show very little selectiveness, and can usually be grown with a large variety of organisms. This selectiveness can be increased or decreased to almost any extent at will. It is increased by repeatedly passing the amebas through the animal organism, and is decreased by prolonged cultivation on artificial media. This last method, however, may increase to some extent the selectiveness of the amebas recently isolated from external sources.

In passing amebas through the animal body, not only do they become more selective, but they acquire a resistance to culture. For example: amebas isolated from tap water grow with a large number of organisms at first. If they are isolated with one variety of microorganism and injected into an animal, an abcess usually follows. As a rule, amebas from the abscess may be grown with the organism introduced. But if the contents of the abscess, or cultures from it, are introduced into a second animal, growth from this animal becomes more difficult and may fail. This difficulty increases with successive animal inoculations, until finally the cultivation of amebas on artificial media may become impossible with the means at command.

Satisfactory symbiotic bacteria include a large number of pathogenic and non-pathogenic organisms. We have devoted especial attention to the colon group, and it is with this class that we have had most success in procuring primary amebic cultures from feces. We have found that organisms which are apparently identical culturally may give different results as symbiotics. For example, bacillus 1650 *b*, belonging to the colon group and isolated from a dysenteric stool, has given us abundant growths where plates, inoculated with organisms indistinguishable from it morphologically or culturally, have failed. We have also been successful with Spr. cholerae and several other vibrios, Staph. pyogenes aureus, B. typhosus, and many unidentified organisms from normal and dysenteric intestines, liver abscesses, air and water. One of these, a yellow pigment producing saprophyte frequently isolated from air and water, has been very useful in animal experiments, since it is easily recognized and is non-

pathogenic for animals as well as man. Other investigators have cultivated amebas upon yeast (Beyerinck), acetic bacteria, B. pyocyaneous, B. rubra, non-sporing bacilli from garden earth, and other bacteria.

The appearance of the amebas in cultures is dependent upon the organism with which they are symbiotic. There are usually no appreciable differences between cultures of amebas from different sources when they are grown with the same organism. Amebas do not colonize or pile up on each other, and they remain upon the surface of cultures.

The course taken by amebas when inoculated in the center of fresh plates is interesting. The amebas move rapidly away from the center, and at first may distance the bacteria. In general the course of the amebas is away from the bacterial masses. The bacteria follow closely, catch up, and pass the amebas when they slacken. Again the amebas forge ahead, only to slacken their speed and be overtaken by the bacteria. Finally the amebas lag behind and become encysted. When active amebas are found on the margins of a plate, the center may be occupied by encysted amebas.

When the amebas of a culture become encysted, no further development takes place until they are transferred to fresh media. These transfers may be successful from one week to at least seven months after encystment takes place.

The round or encysted stage is rare among amebas in their natural fluid environment, and only slightly more frequent in fluid culture of artificial preparation. Encystment may be brought about by changing the reaction of the media from neutral or slightly alkaline to slightly acid, by increasing the nutrition, causing the bacteria to grow more profusely, or by the action of cold, heat, or chemicals.

The morphology of amebas in cultures varies with the age of the parasite, the life cycle, the density of the media, and other factors. Even when taken in the same stage (preferably the round or encysted stage) the measurements of cultures from a dysenteric intestine may vary from 4 to 40μ. Cysts from a single organism apparently vary greatly. After further work the

morphology may become an important point in differentiating species, but at the present time no value can be attached to it.

For staining permanent preparations of amebas from cultures, Wright's modification of the Romanowsky method is the most satisfactory we have yet used.

The means of nutrition of amebas are undetermined. Amebas engulf red blood cells, bacteria, yeast, and other granular material, but it is doubtful if these serve as food. The process of digestion has never been followed out. On the other hand, amebas have been seen to discharge these cells, apparently in a normal condition, after the latter have remained within the protoplasm for some time. In cultures amebas may often be seen to discharge all the granular material and foreign bodies which they contain just before entering the encysted stage.

REACTIONS OF AMEBAS TO VARIOUS AGENTS.

The reaction of amebas to physical agents has been the subject of much study. Celli and Fiocca[1] found that cultures of amebas resisted drying 11 to 15 months, while Miller[2] concluded that some amebas could withstand drying for six years. Our own experiments are incomplete, but we have found that different amebas show different susceptibilities to this agent.

The optimum temperature for the cultivation of amebas is 20° to 28° C. All of our amebas grew profusely at room temperature, slowly in the ice box, and poorly in the incubator. We have been unable to verify the statement that amebas always lose their mobility at or below 75° F. It is certainly not true in the case of cultures. The maximum temperature varies considerably with different organisms. Encysted culture two months old resisted 60° C. for one hour. Others were killed under similar conditions. The minimum temperature also varies with different amebas. Some encysted cultures have been exposed to −12° C. for 45 days and then grew on transplantation.

Sunlight inhibits the growth of amebas; X-rays exert an unfavorable influence on them; and the action of fluorescence causes their encystment and death.

[1] *Centralb. f. Bakt.*, 1894, 15, p. 470. [2] *Ibid.*, 1894, 16, p. 273.

Of the chemical agents acting upon amebas quinine is the most important. If a young slant culture of amebas is washed with a solution of quinine hydrochlorate of a strength of one to 2,500, the amebas quickly encyst and die. The resistance of amebas to alkalies is phenomenal. Three c.c. of $\frac{n}{10}$ KOH can be added to 10 c.c. of neutral media without preventing their growth. Acid, however, readily kills most amebas. Nevertheless some are capable of multiplying in a medium which is more acid than the secretion of the normal human stomach.

We have investigated the action of sera and blood upon amebas. An emulsion of amebas in pure culture with Spr. cholerae was treated with an equal volume of serum possessing a high agglutinating power for the latter organism. The bacteria were very promptly agglutinated. Some amebas became round in a few minutes, others remained active and grew on transplants. A dog received frequent subcutaneous and intraperitoneal injections of amebas and B. coli. After two weeks the serum had no destructive action on the amebas, which grew on plates smeared with the serum. This experiment was repeated with other organisms, with the same result.

Human blood or serum, added in small amounts to fluid culture of an ameba isolated from water caused encystment. Cultures of the same organism on being reclaimed from a liver abscess were not so susceptible to the action of the blood or serum. This susceptibility may be lessened still further by cultivating the amebas on media to which gradually increasing amounts of blood or serum are added.

The amebas we have cultivated from external sources and from the animal organism have been very similar. We judge, however, that there is more than one variety, as we have noted unusual features in two amebas from the amebic colon and in one from the city water supply. If the existence of more than one variety is fully determined, the first ameba shown to comply with Koch's laws should retain the name Ameba coli.

ETIOLOGICAL SIGNIFICANCE

The etiological importance of the amebas in the intestinal and other infections with which they have been associated has been

much debated since the report of Lösch[1] in 1875. This investigator and others who found amebas in dysenteric stools, believed they were the cause of amebic dysentery. Then came the reports of the discovery by Cunningham,[2] Grassi,[3] and others, of amebas in the stools of healthy persons, and of those suffering from other diseases. The conclusion drawn was that amebas probably had nothing to do with the production of the dysentery. The discussion has never been fully settled. The views that have been advocated may be briefly discussed under four headings:

1) Amebas are harmless commensals.

2) Amebas intensify or alter lesions already present in the intestine.

3) There are pathogenic and non-pathogenic amebas.

4) All amebas are, or may become, pathogenic.

The first view, that amebas are harmless commensals, has had many supporters. These claim that amebas are found in the stools of healthy persons. A critical review of the literature, however, does not show conclusively that these individuals were healthy. Most of the reported observations have been determined from single examinations, unconfirmed by the subsequent histories of the patients, and probably none of the cases have been followed long enough. Furthermore, the intestine should not be pronounced healthy at autopsy because no obvious lesions are present, for there is a preulcerative stage in intestinal amebiasis which can be detected only microscopically. Celli and Fiocca report the presence of amebas in three boys in Alexandria and say there was no subsequent history of bowel trouble, but they do not give the period during which these observations were continued. One of Strong and Musgrave's[4] patients remained well for three months and then passed from observation, but this period was too short to show that the bowel was healthy or that the amebas were harmless. We have found that the maximum period of incubation is over five months.

The remarkable latency of this disease was well shown in an examination we made of 300 miscellaneous prisoners. Amebas

[1] *Virchow's Archiv*, 1875, 65, p. 196.

[2] *Quar. Jour. of Micr. Sci.*, 1881, 21, p. 234. [3] *Gaz. Med. Ital. Lomb.*, 1879, 5, p. 451.

[4] *Annual Report of the Surgeon General, U. S. Army*, 1900, p. 251.

were found in the stools of 101 of them. Of these 61 were suffering with dysentery, but the other 40 gave no history of past or present diarrhea. During the next two months, 8 of the 40 died, and each had amebic lesions in the intestine. Of the remaining 32, 15 others developed dysentery, while 17 remained under observation. During the next six weeks 2 of the 17 were discharged, and the remaining 15 developed diarrhea.

Finally, even if we admit, a point unproved as yet, that amebas may be transiently present in the normal colon, or even that they may propagate there, it is not proof that they are harmless. This would leave natural immunity, the influence of environment, and other factors out of consideration.

The supporters of this view also claim that amebas are sometimes present in the intestinal contents of persons suffering from other diseases. In looking over the literature on this point one is struck with three facts: 1) That nearly all of the diseases with which the amebas were associated involved the colon. 2) That most of the observations were made in places where amebic dysentery was endemic. 3) That postmortem observations were rarely made. We have given particular attention to intestinal amebiasis for a number of years in a country where it is endemic. While we have repeatedly found amebiasis associated with a variety of other diseases, we have not found in such observations evidence of the harmlessness of amebas. In a number of cases where amebiasis was associated with cholera and typhoid fever, and in one case of colitis, secondary to carcinoma, the diagnosis of a double infection was established at autopsy.

The second view, that amebas intensify or alter lesions already present, is based upon the finding of amebas in lesions of well recognized etiology; for example, tuberculous ulcers. We can confirm this. In a recent autopsy there were ulcers resembling those of amebiasis, but microscopical and histological study of sections showed the presence of a double infection. Double infections of amebas with diseases producing ulceration of the colon, as typhoid fever, Bright's disease, etc., are not infrequent.

A less tangible argument is the one that amebas are frequently present in the normal intestine and do no harm until "cold,"

indigestion, diarrhea, etc., change the colon and enable the amebas to assume a pathogenic rôle. If amebas are ever present in the normal intestine, this may be true in some cases, as these conditions facilitate the propagation of parasites that reach the intestine during such a time. But there is little to indicate that it is more universally true with amebas than with many other etiological agents.

The third view, that there are both pathogenic and non-pathogenic amebas, usually rests upon supposed differences in morphology and in animal experiments, but this distinction has always been recognized as difficult. In general, the larger amebas, some containing red blood cells, have been considered pathogenic and have been termed Amebae dysenteriae. The smaller species are said not to contain red blood cells, show other morphological differences, are considered non-pathogenic, and are quite generally designated Amebae coli.

Our work has convinced us that the size and other morphological appearances of amebas have little if any relation to their pathogenicity. Some of the most persistent and even fatal cases of dysentery may show amebas of not over 10 to 20 μ in diameter in the stools during life and in the intestinal ulcers postmortem. On the other hand, very large amebas may appear in some of the cases most amenable to treatment and in fatal cases which show the smallest amount of ulceration. In some typical cases of amebiasis, repeated examination of the amebas in the stools failed to reveal blood inclusions. The presence of such cells may merely indicate degeneration of the ameba. In cultures, young active amebas are quite select in their diet, and among other substances avoid red blood cells. But as degeneration sets in, less selectiveness in choice of food is manifested and the protoplasm takes up whatever is within reach.

Animal experiments have also been used to differentiate the pathogenic from the non-pathogenic amebas. But the results of these experiments are so contradictory, even when made with stools known to be dysenteric, that conclusions drawn from them are unwarranted.

We do not deny that there are pathogenic and non-pathogenic

varieties of amebas, but we do desire to say that thus far the existence of such varieties has not been proved, nor are we as yet able to settle the question. Our work has shown, however, that amebas cultivated from various sources, including the dysenteric intestine, the Manila water supply, lettuce, etc., have proved pathogenic under certain conditions.

The fourth view, that all amebas are, or may become, pathogenic leaves much to be explained by natural immunity and other conditions, but it is the only safe hypothesis to adopt in the Tropics. In a country where the parasites are found everywhere it must be true that they are taken daily into the gastrointestinal tract by large numbers of persons. If all amebas are pathogenic, why do we not have more amebic dysentery? If all amebas are not pathogenic, why do we not find them more frequently in the normal intestine? We have never followed a case indicating that non-pathogenic amebas were propagating in the human intestine, and we are sure such cases are rare in the Philippine Islands, and yet the larger part of the population takes in thousands of amebas every day. For we have had no difficulty in cultivating amebas from the very water they use and in producing dysentery in monkeys with the cultures. If both kinds exist, the number of non-pathogenic ones capable of resisting the stomach acids and multiplying permanently in the intestine are very few in comparison with the number of pathogenic ones capable of resisting the same influences and found associated with the lesions of a pathogenic entity.

ANIMAL EXPERIMENTS.

Our experiments with cultures of amebas on animals have been quite convincing as to the etiological role of some amebas. These results have been particularly satisfactory with monkeys. Nothing definite has been gained by working with cats, dogs, and other animals. Even with monkeys infections have not been constant, and the lesions produced in them, while satisfactory, have not been so extensive as those usually seen at necropsy in man. Otherwise the specimens obtained have had the macroscopic appearance of amebic infection, and the parasites have always appeared in the contents of their intestines. A study of

sections from the colon has confirmed the nature of the infection in each instance. In a pneumonia epidemic we were able to study the early lesions of amebiasis in man, and these resembled the lesions in our experimental animals so closely that we are all the more certain of the results of the latter.

Most animals inoculated had been under observation 10 to 30 days. The incubation period in these monkeys was rather long, usually about four weeks, but it was considerably shortened in those that received intra-abdominally, cultures of bacteria that in themselves would cause illness without death. In some cases the onset was rather sudden with severe diarrhea, which continued almost unabated until death, but as in the case with man, it more often started as an intermittent diarrhea which became more severe with each exacerbation. Some monkeys developed diarrhea, but later recovered. Recovery also occasionally takes place in the naturally contracted disease in man. We have seen four such cases, including one unacclimated Caucasian and three natives. The diagnosis in these cases was unquestioned, no treatment was instituted, and a year has elapsed since there were amebas in the stools or clinical evidence of the disease. The other symptoms in monkeys resembled those in man. Anemia and emaciation were constant and appeared early.

In order to secure fresh necropsies, most of our animals were killed, rather than allowed to die, when it appeared that an animal would not live until the following day.

Of more than 100 monkeys kept in the same room with the animals inoculated and under identical surroundings, only three were found with amebic dysentery and two of these were kept in cages beside the experimental animals.

The results in our experiments were obtained by using old encysted cultures of amebas. We have had no success with cultures of young motile amebas. The amebas used had been isolated from several different sources, some from dysenteric stools, others from the city water and from the wash-waters of vegetables. In some cases the dysentery was produced by a single species of amebas in symbiosis at one time with Spr. cholerae and at another with an absolutely non-pathogenic saprophyte, with no appreciable

difference in the results. In some cases the symbiotic organism introduced was reclaimed, in others all attempts to isolate it failed. Some of our experiments may be briefly summarized as follows:

In five cases cultures of amebas were fed to the monkeys. They were introduced through the stomach tube in two cases, once they were given with a high enema, and once the cecum was opened and the cultures placed within it. In all of these cases diarrhea developed. Ulcers were found at necropsy in more than half of the cases.

In four cases cultures were inoculated subcutaneously. Abscesses developed at the point of inoculation in three of these. In the fourth case an abscess containing amebas was found in the lung. Amebas were present in three abscesses and they were cultivated from two of them.

Intra-abdominal injections were made in four cases. In only one of them did the intestine show lesions. Abscesses developed at the point of inoculation in two cases, and the fourth case was negative.

In three cases injections were made into the liver. In two of these amebic abscesses formed in the liver, and from one of them the amebas were cultivated. In the third case, the monkey was first rendered actively immune to Spr. cholerae before making the injection of the amebas in symbiosis with a pure culture of Spr. cholerae. At necropsy the liver was normal and neither the amebas nor the Spr. cholerae could be cultivated from it.

We made one experiment upon a human being. A healthy man was kept under observation for ten days, during which time his stools were repeatedly examined microscopically and culturally after the administration of cathartics, but no amebas were found. The man then ingested three ordinary gelatin capsules which contained scrapings from a three week old culture of an ameba isolated from a dysenteric stool. This culture was growing in symbiosis with a non-pathogenic bacterium.

Twelve days later there was a slight diarrhea and amebas appeared in the stool. A mild intermittent diarrhea continued until the 20th day, when some tenesemus was complained of and the stool contained considerable mucus and a small amount of blood in addition to a large number of amebas, some of which enclosed red blood cells. This evidence was considered sufficient to establish the diagnosis, and the patient was placed under treatment. Both the ameba and the bacillus introduced were reclaimed by culture on the day after the development of the diarrhea.

While the evidence now at hand does not warrant us in con-

cluding that all amebas are pathogenic, still all the known facts about the infection are compatible with this theory.

EXPLANATION OF PLATE 12. (Photomicrographs by Martin.)

1. A plate culture showing the spread of amebas and bacteria. The amebas are seen as white spots in the margins and points of the more homogeneous bacterial growth. The peculiar character of the spread of the combined growth is due to the wanderings of the amebas and the growth of the bacteria carried along by them. A 24-hour plate culture of ameba 11524 in symbiosis with B. coli. (Zeiss obj. AA, ocular No. 4; bellows 30 c.c.)

2. An eight-day old plate culture, showing the agglomeration of amebas sometimes seen in cultures. The amebas are all in the round stage. Same enlargement as Fig. 1.

3. A cover-glass impression from two 24-hour old plate cultures of ameba 11524, stained by the Wright-Romanowsky method. × 900.

4. A cover-glass impression from a 24-hour old plate culture of ameba 11524 in symbiosis with bacillus 12935, stained by the Wright-Romanowsky method. The nucleus and the so-called spores and vacuoles are distinctly shown, as is also a granular sppearance of some of the so-called spores. × 2000.

PLATE 12.

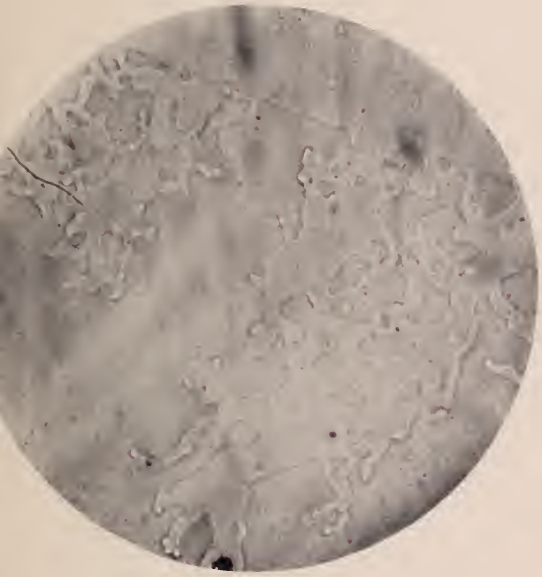


FIG. 1.

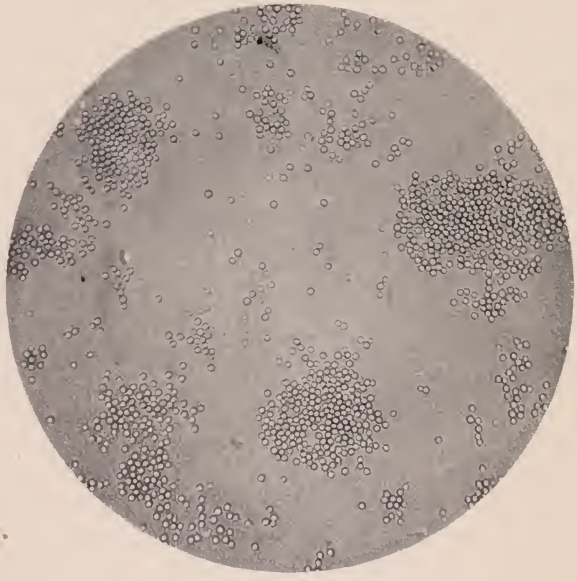


FIG. 2.

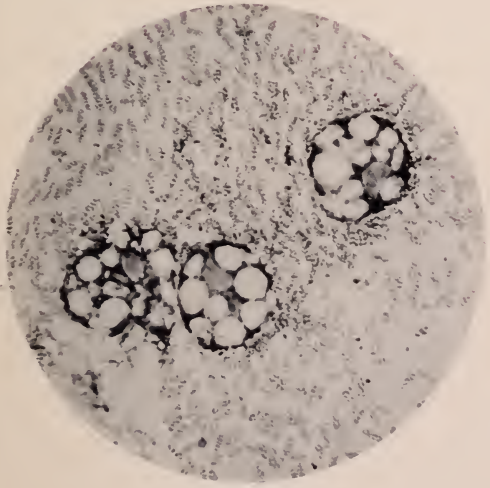


FIG. 3.

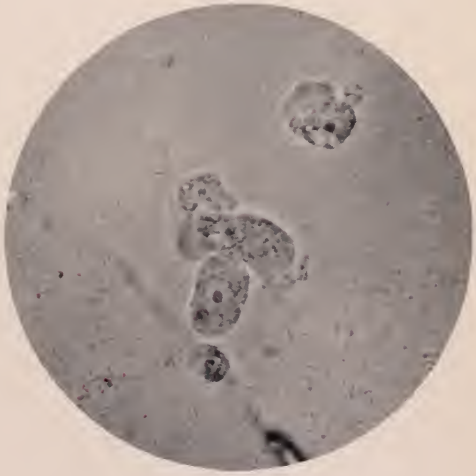


FIG. 4.

STUDIES ON HOG CHOLERA.*

CHARLES T. MCCLINTOCK, M.D., CHARLES H. BOXMEYER, A.B., AND J. J. SIFFER, M.D.

(From the Department of Experimental Medicine, Parke, Davis & Co., Detroit.)

I.

THE VALUE OF AGGLUTINATION FOR THE DIAGNOSIS OF HOG CHOLERA.

CHARLES T. MCCLINTOCK, CHARLES H. BOXMEYER AND J. J. SIFFER.

The close resemblance of the hog cholera to the typhoid bacillus, and the somewhat similar symptoms and course of the diseases caused by them, suggested that agglutination might prove as valuable a method of diagnosis for hog cholera as it has proven for typhoid. The first application of the agglutination test for this purpose was made by Dinwiddie,[1] of the Arkansas Experiment Station. The few cases he tested all gave a negative reaction. No hog cholera bacilli were isolated from the outbreak which furnished this material. Dawson,[2] Smith,[3] and others have made experiments with the blood of vaccinated rabbits which show the reaction often in high dilutions. We are unaware of any other experiments than those of Dinwiddie with the blood of diseased hogs.

The material with which we worked was obtained from the following seven epizootics:

1. *Hastings, Mich.*—The disease first appeared in slaughter-house hogs fed on refuse. It was communicated by them to hogs on an adjoining lot separated only by a wire fence. From there it spread to neighboring farms. Practically all the animals exposed contracted the disease. The mortality, including animals of all ages, was 50 to 60 per cent. The animals that survived apparently thrived. Autopsy showed considerable variation in the pathological appearances of the individual animals examined. Practically all the lesions described for hog cholera were found. The intestinal changes as

* Received for publication January 14, 1905.

[1] *Jour. Comp. Med. and Vet. Arch.*, 1900, 21, p. 528.

[2] *New York Med. Jour.*, February 20, 1897, 65, p. 253.

[3] *Jour. Med. Research*, 1903, 91, p. 270.

a rule were quite marked. A marked hyperemia only; small deep ulcers, large diphtheric patches, areas denuded of epithelium, a diphtheric membrane covering the entire cecum and colon, raised button ulcers, fungoid growths, and follicular abscesses were observed. Pneumonitis, pleuritis, and pericarditis were frequently found. Edematous and hemorrhagic lymph glands were practically constantly present. The spleen was usually small and the kidneys showed a pale cortex, frequently with hemorrhagic spots. These latter were variations from the usual description of hog cholera. Swine plague bacilli were obtained from the hogs showing lung lesions.

2. *St. Johns, Mich.*—Hog cholera had been prevalent in this locality for several years. From July to November two townships had lost about 1,000 head. The loss was about 60 per cent. The presence of a severe diarrhea characterized this outbreak. Autopsy on three animals showed lesions similar to those seen in the Hastings outbreak. Lung and intestinal lesions were both present.

3. *Chatham, Ont.*—Here also the disease had existed for several seasons. During the summer severe losses had occurred. In December, at the time we visited the locality, the disease had ceased to spread. The mortality exceeded 50 per cent. On account of the severe cold weather and the Dominion quarantine laws, but two autopsies were made. Lung lesions were most marked. The Canadian inspectors spoke of intestinal and foot lesions as occurring.

4. *Belle Plaine, Ia.*—George W. Dunphy, D. V. S., described this outbreak as follows: "This is a very virulent form of a mixed hog cholera and swine plague, destroying more than 90 per cent of each herd in which it appears. In the first autopsies only swine plague lesions were found, but later I discovered well marked hog cholera lesions. In the first postmortems the stomach and intestines were practically normal. The lungs were completely hepatized. On section pus exuded from the bronchi and a bloody fluid from the lung tissue. The liver and spleen were normal in appearance. In the later postmortems I found extensive congestion, ecchymoses and thickening of the intestinal mucosa, also the characteristic projecting button ulcers of hog cholera. In the very acute cases there were extensive intestinal hemorrhages, and large blood clots were found among the intestinal contents."

5. *Lincoln, Neb.*—This outbreak occurred at the Nebraska Experiment Station. The disease was introduced by a pig purchased at the State Fair. A heavy loss occurred. Dr. Dunphy described the lesions as similar to those found in the Belle Plaine, Ia., epizootic. Both intestinal and lung lesions occurred.

6. *Tekonsha, Mich.*—Two hogs shipped by Dr. Dunphy to the laboratory were killed and examined. Both showed follicular abscesses in the large intestines, and hemorrhagic lymph glands. The other organs were apparently normal. These were convalescent cases. Hog cholera bacilli were not found.

7. *State Insane Asylum, Pontiac, Mich.*—This is the outbreak which is described in the succeeding section on "The Production of Hog Cholera in Swine by Inoculation with Bacteria-free Filtrates," *q. v.*

Bacteriological examinations were made of a number of animals, as follows:

	Animals
Tekonsha, Mich.	2
Hastings, Mich.	9
St. Johns, Mich.	4
Pontiac, Mich.	4
Belle Plaine, Ia.	2
Lincoln, Neb.	1
Chatham, Ont.	1

From viscera from Belle Plaine, Ia., and Lincoln, Neb., hog cholera bacilli were isolated which agreed quite closely with each other in cultural and agglutinative characters. These bacilli were but moderately virulent for experimental animals.

From the Pontiac outbreak we isolated an organism of the hog cholera group apparently identical in morphological, cultural, and agglutinative characters with the bacillus β of swine dysentery of Theobald Smith.

In no other instance were we able to isolate true hog cholera bacilli, although a number of organisms quite closely related were found.

TECHNIQUE.

Suspensions of agar cultures 16 to 24 hours old in salt solution saturated with tri.- chlor.- tertiary- butyl-alcohol (chloretone) were employed throughout this work. Dilutions of the serum to be tested were made in physiological salt solution, and equal parts of the serum dilution and the bacterial suspension mixed in small test tubes (8x80 mm.) The tubes were then placed in the incubator at 37° C. They were examined first at the end of two hours and again after 16 or 20 hours. The highest dilutions in which plain floccules showed were taken as the limit. Although the bacterial suspension remained fit for use for many weeks, fresh suspensions were usually made for each test. Control tests with young broth cultures and the microscope were made from time to time. In a number of instances dried blood was used; this we found reacted fully as well as the fresh serum.

THE PRESENCE OF AGGLUTININS FOR HOG CHOLERA BACILLI IN THE BLOOD OF NORMAL HOGS.

The blood of normal hogs usually agglutinates hog cholera bacilli in fairly high dilutions. For our purpose it was necessary to determine the normal range. Of 22 specimens of normal hog blood examined 3 agglutinated hog cholera bacilli in dilutions of

1–200.* 11 in dilutions of less than 100; the remainder between these limits. (The smaller amount of agglutinin is usually found in the blood of the younger hogs.) The blood of normal hogs agglutinates not only the hog cholera bacillus but also others: swine plague, colon and paracolon bacilli in dilutions of about the same strength as the hog cholera organism.†

From a consideration of these data it was concluded that an agglutination in a dilution of less than 1–300 is of no value in the diagnosis of an infection by hog cholera bacilli.

AGGLUTINATIONS WITH THE BLOOD OF HOGS SICK WITH OR DEAD OF HOG CHOLERA.

The blood of diseased hogs in the outbreaks studied reacted only occasionally with hog cholera bacilli in dilutions of 1–300. The following table gives the results of the examination of the blood of 57 different hogs from the several outbreaks:

TABLE I.

THE AGGLUTINATION OF HOG CHOLERA BACILLI BY THE BLOOD OF DISEASED SWINE.

Source of Blood	No. Animals from which Blood was Tested	Reaction and Dilution	Hog Cholera Bacillus Isolated
Hastings	7	— 200	no
St. Johns	3	— 300	no
Takonsha	2	— 250	no
Belle Plaine			
Herd 1	7	— 300	
Herd 2	3	— 300	
Herd 3	4	+2000	yes
	2	+1000	
Lincoln	5	+ 600	
	15	— 300	yes
Pontiac	7	— 50	
	1	+ 800	yes
Chatham	1	— 300	no

In those instances where a hog cholera bacillus was isolated from an outbreak, the agglutinating power of the blood of the animals from this locality was tested against this particular bacillus. In those outbreaks from which no hog cholera bacillus

*That the presence of this relatively large quantity of agglutinin cannot be explained by the animals having had a previous attack of the disease is shown by their succumbing to hog cholera when subsequently exposed.

†The Pontiac bacillus was never agglutinated by normal hog blood in any of the trials made in dilutions of 1-50.

was isolated the agglutinative power of the blood was tested upon several strains of hog cholera bacilli.

Table I shows plainly that agglutination is of no value for the diagnosis of hog cholera, as the disease is at present defined. The presence of a positive reaction, if obtained in dilutions of 1–300 or over, does, however, indicate an infection with hog cholera bacilli.

II.

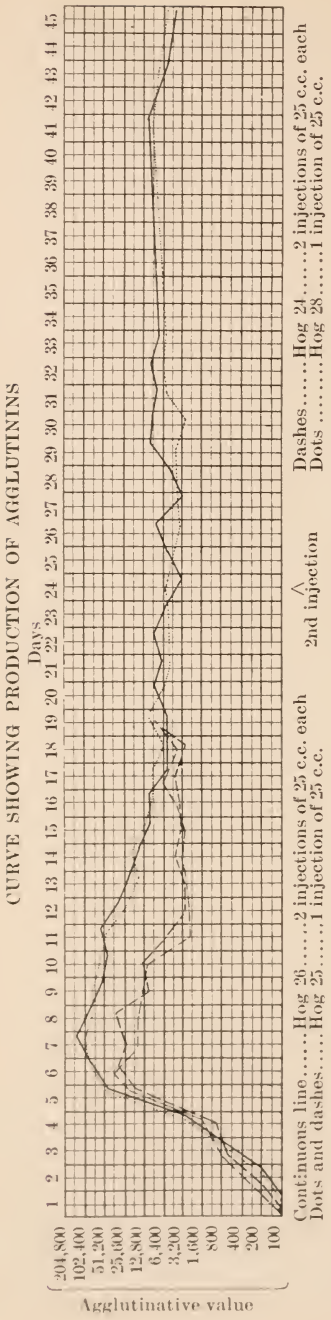
THE PRODUCTION OF AGGLUTININS FOR HOG CHOLERA BACILLI IN SWINE.

CHARLES T. MCCLINTOCK, CHARLES H. BOXMEYER AND J. J. SIFFER.

AGGLUTINATION AFTER VACCINATION WITH HOG CHOLERA VACCINE.

The vaccine used in these experiments was prepared by growing 11 different strains of hog cholera and one of swine plague bacilli upon agar in quart whiskey flasks. After incubating at 37° C. for 60 hours the growth was loosened from the agar surfaces with a bent glass rod and washed off with a .75 per cent salt solution saturated with tri.- chlor.- tertiary- butyl-alcohol. The suspension was then strained through India mull to remove particles of agar and cotton, the several strains mixed, and heated to 50° C. for 30 minutes. The vaccinations were practically always made intraperitoneally. The animal was bled seven or eight days afterwards from the tail or ear.

Most of the animals reacted promptly to the injection producing relatively large quantities of agglutinin. A series of four hogs was vaccinated with 25 c.c. of hog cholera vaccine and the agglutinating power of their blood tested each day. The amount of agglutinin in the blood began slowly to rise on the second and third day, then it rose rapidly till the sixth day when it attained its maximum. It stayed at this point for about three days. During the succeeding week it dropped either suddenly or gradually to a relatively constant level at which it remained for some time. A second vaccination 24 days after the first raised the level slightly, but it never attained its former maximum height. This is not



true, however, in all cases, the blood of some hogs showing the highest agglutinating power after repeated injections.

Twenty-six vaccinated hogs, bled seven days after injection, showed the following maxima:

3 hogs	75,000	to	100,000
1 hog	50,000	to	75,000
3 hogs	20,000	to	50,000
6 hogs	10,000	to	20,000
7 hogs	5,000	to	10,000
6 hogs	1,000	to	5,000

The quantity of agglutinin produced varied somewhat with the lot of the vaccine used. The size of the dose, however, appears to have had no influence upon the amount of agglutinin produced, this depending apparently upon an idiosyncrasy of the animal.

TABLE II.

RELATION OF AMOUNT OF VACCINE TO HEIGHT OF AGGLUTINATION.

No. of Hogs	Amount of Vaccine	Lots of Vaccine	Agglutination Limit Seven Days after Injection
1.....	25 c.c.	.05183	50,000
1.....	25 "	.05183	10,000
1.....	25 "	160 H	5,000
1.....	25 "	160 H	2,000
1.....	20 "	.05072 B	1,200
1.....	20 "	.05072 B	300
3.....	12 "	160 H	10,000
1.....	12 "	160 H	20,000
1.....	12 "	160 H	7,500
1.....	10 "	160 H	15,000
1.....	10 "	160 H	5,000
1.....	10 "	.05072 B	1,000
1.....	10 "	.05072 B	300
2.....	5 "	160 H	10,000
1.....	5 "	160 H	5,000

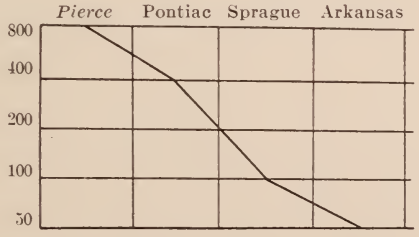
In a few cases agglutinins had not appeared in the blood seven days after vaccination.

Hogs inoculated with mixed hog cholera and swine plague vaccine show in their blood an agglutinin for the swine plague as well as the hog cholera bacillus.

Two hogs with an agglutinating limit before treatment of 50 and 75 for swine plague bacilli and 100 and 150 respectively for hog cholera bacilli, showed seven days after vaccination with mixed vaccine an agglutination of 1–500 and 1–10,000 for swine plague, and 1–15,000 and 1–50,000 for hog cholera bacilli.

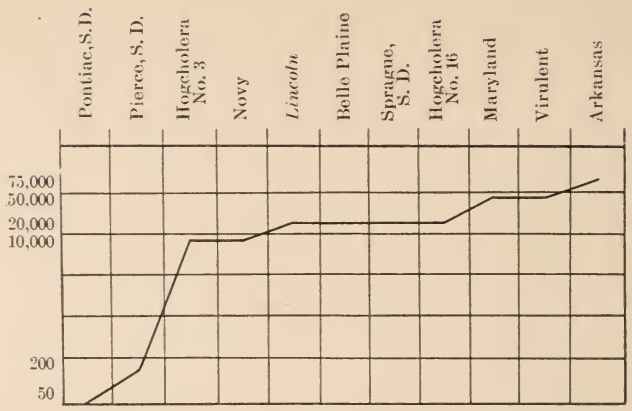
AGGLUTINATING POWER OF THE BLOOD AFTER INOCULATION WITH LIVE HOG CHOLERA BACILLI.

Hog No. 103, weighing 75 pounds, received into the ear vein six c.c. of an 18-hour culture of a hog cholera bacillus obtained from the Lincoln (Nebraska) outbreak, but a few weeks after its isolation. The animal was bled to death on the 13th day. The following curve shows the agglutinating action of its serum upon various strains of hog cholera bacilli:



A hog injected subcutaneously with five c.c. of the Pontiac bacillus agglutinated seven weeks afterward the injected bacillus in a dilution of 1–800, but did not agglutinate the Arkansas bacillus in a dilution of 1–50.

The following curve of agglutination by the serum of a rabbit inoculated with the Pontiac organism shows graphically the agglutination relations of the hog cholera and swine dysentery bacilli:



MAY HOG CHOLERA BACILLI BE PRESENT IN THE ORGANISM AND THE AGGLUTINATION REACTION REMAIN NEGATIVE?

Case 1.—Hog No. 106 was fed viscera of hogs in which the Arkansas hog cholera bacillus was present. He died seven days afterward. From the spleen, liver, and mesenteric glands pure cultures of the same hog cholera bacillus were obtained. The blood serum of this animal gave no reaction either with the bacillus isolated or with the original culture. This observation was confirmed by a repetition of the test.

Case 2.—Hog No. 105 received intravenously six c.c. of the juice of the organs of hog 48, filtered through paper. He was dead on the 13th day. Autopsy showed typical swine plague lesions. Cultures from the various organs gave a mixture of the swine plague and hog cholera bacillus.*

The blood serum gave no agglutination in dilutions of 1–300 with the hog cholera bacillus isolated.

The blood serum of two hogs from the Pontiac outbreak, from which hog cholera bacilli were isolated, gave no agglutination either with the Pontiac organism or strains of ordinary hog cholera bacilli in dilutions of 1 to 50.

*The Arkansas bacillus, which was kindly sent us by Dr. Theobald Smith, is admirably adapted for experimental work on account of the readiness with which it may be identified. It differs markedly in the appearance of its colonies from all other strains we have studied. On broth it forms a thin, white, brittle membrane. On agar it forms characteristic dry, slightly wrinkled colonies, readily detached in one piece from the agar surface. It is difficult to break up the growth and make up a homogeneous suspension. Its action upon various culture media and toward agglutinating serum was almost identical with that of the other strains in our possession. The peculiarities were retained through repeated animal passages.

SUMMARY.

1. The serum of normal hogs agglutinates strains of ordinary hog cholera bacilli in dilutions occasionally as high as 1 to 250. For this reason we consider a reaction in a dilution of less than 1 to 300 without diagnostic value.

2. The bacillus of swine dysentery is not agglutinated by normal blood in such high dilutions.

3. Agglutination is of no value for the diagnosis of hog cholera, as the disease is at present defined.

4. The presence of a positive reaction does, however, indicate an infection with hog cholera bacilli.

5. There are occasional instances of both natural and artificial infection in which no increase of the agglutinins for hog cholera over those normally present can be demonstrated.

6. The maximum amount of agglutinin develops in a hog's blood within six or seven days after a single inoculation with hog cholera vaccine.

7. Hogs react to intraperitoneal injections of hog cholera vaccines, usually with the production of large quantities of agglutinins, the amount of the vaccine bearing no relation to the amount of agglutinin produced.

III.

THE PRODUCTION OF HOG CHOLERA IN SWINE BY INOCULATION WITH BACTERIA-FREE FILTRATES.

CHARLES H. BOXMEYER.

Repeated failures, after thorough and prolonged search, to isolate hog cholera bacilli from the carcasses of hogs which had succumbed to the disease in certain Michigan outbreaks, the failure of the blood serum of any of these animals to agglutinate any of several hog cholera strains, together with the inability to produce the characteristic lesions of hog cholera by the feeding or injection of hog cholera bacilli, led to a search for some other agent as the etiological factor of these outbreaks.

Opportunely, the Bureau of Animal Industry[1] at this time

[1] DESCHWEINETZ and DORSET, *Bureau of Animal Industry*, 1903, Circular 41.

published a preliminary report upon "A Form of Hog Cholera Not Caused by the Hog Cholera Bacillus," in which they stated that they were able to transmit the disease to healthy animals "by the subcutaneous inoculation of certain body fluids, these fluids being always proved by careful bacteriological examination, by filtration through the finest porcelain filters, and by the inoculation of guinea pigs and rabbits, to be free from hog cholera and swine plague bacilli."

Experiments were immediately begun along this line, as the confirmation of this work would explain the failure of all attempts to immunize hogs by means of vaccines or sera against hog cholera, and point out the way to a possibly successful method of combating this disease.

EXPERIMENTS WITH FILTERED BODY FLUIDS.

The infectious material employed was derived from an epizootic of hog cholera that occurred at the State Insane Asylum at Pontiac, Michigan, in May, 1904. At the time of investigation the disease had been current for about six weeks. The herd had originally contained 90 head—Berkshires, Chester-whites, and crosses—weighing from 100 to 200 pounds. Fifty deaths had occurred, and practically all the remaining hogs showed evidence of a more or less advanced stage of the disease. The symptoms shown were fever, reddening of the ears and skin covering under surface of the body, partial paralysis of the posterior extremities, and eyelids swollen and glued together. Some animals exhibited a marked constipation, while others showed a severe diarrhea. The superintendent kindly permitted us to kill two of the sickest animals for postmortem examination. Autopsy showed the following:

Hog No. 1.—Chester-white, male, weight 100 pounds. Had shown disease symptoms for about one month. Condition fair. Weakness of the hind legs. Killed and examined. Skin shows slight flush. Inguinal glands not enlarged. Spleen hyperplastic. Liver pale, of a yellowish color. Kidneys with pale cortex dotted with minute petechiæ. Mesenteric glands edematous and hemorrhagic. Cecum and colon filled with hard, impacted feces. Mucosa dark colored. Two indurated ulcers about 15 m.m. in diameter at the base of the ileocecal valve. Echinorhynchi and ascarides present. Small intestines little changed. Apices of both lungs hepatized, with pleural adhesions at these points. Visceral pericardium petechiated.

Hog No. 2.— Berkshire, weight 125 pounds. Marked weakness in both hind and fore legs. Condition fair. Killed and examined. Inguinal lymph glands not enlarged. Spleen slightly enlarged and covered with fleshy villi. Kidneys with pale cortex sparsely petechiated. Liver pale. Intestinal mucosa shows no marked change, and intestines are filled with a large quantity of semi-fluid feces. Lungs show fairly large sub-pleural ecchymoses. The posterior lobe of left lung is congested and shows beginning of hepatization. Lymph glands swollen and slightly hemorrhagic.

Cultures from the various viscera gave a variety of organisms, from which we were able to isolate a hog cholera-like bacillus, identical in morphological, cultural, and agglutinating characters with the bacillus of swine dysentery β of Theobald Smith. One-half c.c. of a 24-hour culture of this bacillus injected subcutaneously into a rabbit produced death in six days. Postmortem showed the focal necroses of the liver characteristic of an infection by members of the hog cholera group. The blood serum of this rabbit agglutinated the injected bacillus and the swine dysentery bacillus β of Smith in a dilution of 1–800, but produced no agglutination in dilutions of 1–50 with the ordinary strains of hog cholera bacilli. Five c.c. of a 16-hour broth culture of this bacillus in the fourth generation, and four days after isolation, produced no other effect than a considerable local abscess when injected subcutaneously into a 20-pound hog during the 30 days he was under observation. The animal gained rapidly in weight during this period.

Portions of the spleen and liver of the above hogs were brought to the laboratory and fed to hog No. 114, a Chester-white pig, weighing 20 pounds. He was chloroformed on the 29th day after infection, being moribund. The blood was collected aseptically from the carotid artery.

Postmortem: Great emaciation. Ears and snout blackened and necrotic. Feces thin, yellow, fluid. Inguinal glands enlarged, not hemorrhagic. Spleen hyperplastic and bound to the cecum by dense adhesions. Liver yellowish. Kidneys pale, with a few minute petechiæ. Mesenteric and gastric glands enlarged and hemorrhagic. Small intestines little changed. Colon and cecum show hemorrhagic areas on their serous surface. Mucosa thickened and covered throughout with a diphtheritic layer yellowish in places, black in others. Button ulcers show above this membrane. Lungs slightly congested. Tracheal glands hemorrhagic. Pure cultures of bacillus pyocyaneus were obtained from the spleen, liver, kidney, and mesenteric glands.

SECOND PASSAGE. FIRST FILTRATE.

The blood of this hog (No. 114) was mixed with two parts of sterile physiological salt solution and filtered through a large Berkefeld filter (Laboratory Cylinder No. 2), using suction. The filtrate was removed from the sterile suction flask by means of a sterile pipette, filled into a sterile syringe, sealed, immersed in five per cent carbolic acid solution, and wrapped in cotton moistened with the same disinfectant. So wrapped, the syringe was handed to a second person, who had never been near the diseased hogs, nor in the room where the filtration took place. This person made the injection. The animal chosen was a Chester-white pig, No. 117, weighing 25 pounds. It received (June 17) intraperitoneally 10 c.c. of the above filtrate, equivalent to

3.3 c.c. of undiluted serum. The animal was placed in an isolated pen at a distance from the diseased hogs. The stableman who attended him never came in contact with diseased hogs nor their attendant. The same attendant cared for some uninoculated hogs during this time, which remained well during the experiment, but later succumbed to the disease after a normal incubation period when exposed to infection. The animal remained active, but lost weight, and became much emaciated. A few days before death, he showed discolored skin, tottering gait, and blood-stained urine. He died during the night of July 11, 24 days after inoculation.

Postmortem: Body emaciated. Skin discolored and scabby. Inguinal and cervical lymph-glands swollen and intensely hemorrhagic. Numerous small subcutaneous hemorrhages. Peritoneal cavity contains considerable bloody serous exudate, in which are particles of fibrin. The bladder has the appearance of a large blood-clot; the walls are greatly thickened and infiltrated with blood. Both large and small intestines show numerous hemorrhagic spots on the serous surface. The mucosa of colon and cecum show numerous large yellowish button ulcers with black centers. These ulcers are occasionally confluent. The lungs show numerous petechiæ and a nodule of walnut size. Heart pale and flabby. Visceral pericardium dotted with many small ecchymotic spots.

Portions of the filtrate with which the above hog was injected were tested, as follows:

Five c.c. was added to 500 c.c. of broth.
Two c.c. was added to 500 c.c. of broth covered with oil.

The first flask showed, after some days, a slight cloudiness. Subcultures and injection of 2.5 c.c. of the flask contents subcutaneously into a rabbit failed to show any bacteria. Microscopic examination showed numerous minute granules adhering together in clumps. These were difficult to stain with the ordinary anilin dyes, and probably were precipitated albuminous substances.

A guinea pig (300 grams) received seven c.c. subcutaneously without effect.

A mouse (20 grams weight) received two c.c. subcutaneously. No effect.

Rabbit No. 117, five c.c. subcutaneously, without effect.

Rabbit No. 116, five c.c. intravenously. Died on the 39th day from pneumonia. The animal showed signs of snuffles before death. Bipolar staining bacilli were found in large numbers in the pus from pleural exudate.

THIRD PASSAGE, SECOND FILTRATE.

The blood and peritoneal fluid of hog No. 117 were left in the refrigerator for 11 days, then diluted with three parts of sterile salt solution, filtered first through paper, then passed twice through a large Berkefeld filter and finally through a Chamberland Pasteur F filter using suction. This filtrate was filled into a syringe, sealed and handled under the same precautions as the first filtrate. Cultures on agar and broth gave no signs of growth. Rabbit No. 121 received subcutaneously nine c.c. of this filtrate without result. Rabbit No. 120, five c.c. intravenously without effect.

Mouse No. 150, (15 grams) one c.c. subcutaneously. Found dead on the second day. No bacilli could be found at the point of inoculation upon microscopic examination.

Hog No. 124, Chester-white, weighing 20 pounds, received subcutaneously 20 c.c. (equal to five c.c. serum) of the filtrate from hog No. 117. This animal was kept in a dog-cage in an animal room at a distance from the diseased hogs, and neither the person who injected the animal nor the attendant ever came in contact with any diseased swine, nor into the room where infectious material was handled. No one that had handled infected material entered the room during the period of isolation. The animal showed on the 11th day distinct signs of disease, reddening of the skin, loss of appetite, and intense thirst. On the 10th day a marked diarrhea set in and the pig lay crouched in the corner of the cage from which it refused to move. On the 17th day after inoculation, the animal, being apparently moribund, was chloroformed and bled from the carotid artery, the blood being collected aseptically.

Postmortem: Much emaciated. Skin discolored. Ears purplish. Inguinal glands greatly swollen with some hemorrhagic lobules. Spleen normal, liver normal. Cortex of kidneys pale with a few petechiae. Mesenteric and retroperitoneal glands swollen and slightly hemorrhagic. Pancreas normal. Colic and cecal mucosa free from ulcers, but dotted with exceedingly numerous minute hemorrhages. Lungs with small sub-pleural hemorrhages. Post-tracheal glands hemorrhagic and edematous. Blood smears show a considerable number of nucleated red corpuscles.

Cultures were made from the spleen, liver, kidneys and lymph glands. Broth tubes from the first two showed a few large cocci. The others were sterile.

The blood serum of this animal failed to agglutinate in 20 hours either the Pontiac swine dysentery or a typical hog cholera bacillus, in dilutions of 1–100. Cultures and inoculations of rabbits made with the blood serum of this hog showed no bacteria.

FOURTH PASSAGE. UNFILTERED SERUM.

Since the first two passages and filtrations should have eliminated all filterable organisms, and as the severity of the lesions was apparently decreasing it was decided to return to unfiltered serum in hopes that the more characteristic lesions of the disease would reappear.

Hog No. 134, Berkshire, weight 20 pounds. Received subcutaneously five c.c. of the unfiltered sterile serum of hog No. 124. The same precautions in regard to the syringe and the isolation of the animal were observed as before. On August 26, the 13th day after inoculation plain signs of the disease were present. The animal died September 6, 24 days after inoculation.

Postmortem: Slight loss of flesh. Redness of skin of abdomen and chest Inguinal lymph glands enlarged and hemorrhagic. Considerable quantity of fluid exudate in the peritoneal cavity. Intense fibrinous peritonitis, thick layers of fibrinous exudate matting together the viscera and covering liver and spleen. The whole length of small intestines hemorrhagic, mucosa hyperemic. Cecum and adjoining portions of colon show numerous round

depressed ulcers from three to eight mm. in diameter. A circular ulcerated ring around the ileocecal valve. The remaining portion of the large intestines shows scattered hemorrhagic areas. Spleen slightly enlarged. Liver and kidneys apparently normal. Fibrinous pleuritis and pericarditis. Parietal pericardium adherent to the heart. Small congested and edematous areas in the apices of the lungs, other portions normal. Posttracheal glands enlarged and hemorrhagic.

Cultures made from liver, spleen and kidneys all showed growth. None of the organisms obtained were agglutinated either by a hog cholera or a swine dysentery serum.

In addition to the above series a small white hog No. 116, weight 20 pounds, was inoculated with a filtrate made by steeping the viscera brought from Pontiac in salt solution, passing through paper and a Berkefeld filter. The first two weeks he apparently gained in weight, but later appeared dull, sniffled and lay in the corner of the cage. He died on the 35th day after inoculation. The precautions against accidental infection were the same as observed before.

Postmortem: Reddening of the ears. Inguinal glands greatly enlarged with minute punctiform hemorrhages. Cervical lymph glands enlarged and hemorrhagic. Spleen dark, not enlarged. Liver normal. Small fibrinous particles adhered to the spleen and serus covering of the intestines. Mesenteric glands enlarged. Gastric and mesocolic glands hemorrhagic. Small intestines filled with yellow fluid feces. Kidneys pale with a few hemorrhagic spots. Large intestines practically normal except at the base of the ileocecal valve where were found two small yellowish ulcers. Apices of both lungs hepatized. Post-tracheal lymph glands edematous and hemorrhagic. Considerable blood-stained purulent exudate. The Pontiac bacillus was isolated from the mesenteric glands of this hog.

Early in the winter some preliminary experiments had been made with infectious material from the outbreak that occurred at Hastings, Michigan, in September, 1903.*

Neither in material from this epizootic brought from the field nor in the animals infected in the laboratory could hog cholera bacilli ever be isolated, nor did the blood serum of any of these animals exhibit any agglutinating power for hog cholera bacilli, in excess of that normally present.

The liver and spleen of hog No. 47 (an acute hemorrhagic case produced by feeding viscera brought from Hastings) were steeped in salt solution and the fluid filtered first through paper, then through a No. 6 Berkefeld filter.

*This epizootic was complicated in one herd by foot lesions from which an anërobic bacillus, a member of the malignant edema group, was isolated. The disease resembled blackleg in being usually confined to one-quarter of the body. The lesions consisted of a hemorrhagic edema with emphysema extending along the limb into the foot. Ulcers were present on the foot and between the toes. In one animal the entire liver was emphysematous, and had the appearance and feel of lung tissue. A rabbit inoculated with edema fluid from this animal showed a similar condition of the liver. These lesions were reproduced in the laboratory in hogs fed infected carcasses. The bacillus died out before it could be more carefully studied.

Hog No. 62, a Chester-white, weighing 30 pounds, received intraperitoneally four c.c. of the above filtrate. No bacteria were detectable in this filtrate by cultures or animal inoculation. Fifteen days after inoculation, the pig showed diarrhea and blood-stained feces. Plates (Drigalski & Conradi) made from the feces failed to reveal hog cholera bacilli. Death occurred in 38 days. Autopsy showed discoloration of the skin, hemorrhagic lymph glands, small dark spleen, ecchymotic mucous and serous membranes, and the colon and cecum had scattered thickly throughout their length small yellowish nodules about the size of a goose shot. These nodules were due to the distention of Klein's glands by cheesy pus. Cultures showed a variety of bacilli but no hog cholera bacilli could be isolated.

Hog No. 58, Chester-white, 30 pounds, received intraperitoneally two c.c. of the edema fluid of hog No. 47, filtered through a small Berkefeld filter. Cultures and animal inoculations of the filtrate showed no bacilli. The course of the disease was similar to that of hog No. 62. The animal died on the 38th day after inoculation. The pathological appearances were similar to those in hog No. 62. The colon and cecum showed numerous raised button ulcers 6 to 20 mm. in diameter scattered along their length, and a large necrotic patch at the ileocecal valve.

Hogs No. 109 and No. 110, weighing 25 pounds each, received subcutaneously respectively 14 c.c. of a Berkefeld No. 2 and 10 c.c. of a Pasteur F. filtrate of an emulsion of the viscera of hog No. 106 (infected with material from hog No, 47). Hog No. 109 died on the 20th and hog No. 110 on the 24th day after infection. No characteristic lesions were present. Both showed slight pneumonia and hyperemia of the intestinal mucosa. Cultures from hog No. 110 gave no growth. Hog No. 109 gave pure cultures of streptococci. The blood of neither hog agglutinated hog cholera bacilli in dilutions of 1–300.

In summarizing the data we find that of five hogs inoculated with sterile Berkefeld filtrates derived from two distinct outbreaks, all died within a period varying from 19 to 38 days, a period which coincides with that observed in cases of natural infections and those produced by feeding infected material. Four of these (Nos. 62, 58, 114, and 116) showed the characteristic intestinal ulcers of hog cholera. Both hogs (Nos. 110 and 124) inoculated with Pasteur filtrates died, one on the 24th, the other on the 19th day after inoculation. Though the source of the infecting filtrate was different (one Hastings, the other Pontiac), both showed similar lesions, which, while not characteristic of hog cholera are frequently found in natural infections. The blood serum of one of these (hog 124), when injected into another hog (No. 134), produced the characteristic lesions of hog cholera, the animal dying in the usual period, namely, 24 days.

The above observations are in agreement, on the whole, with those of the Bureau of Animal Industry, but differ in that we had to deal with the chronic form of the disease, while their virus always gave rise to the acute. In an attempt to produce an acute infection a 100-pound hog received an injection of five c.c. of virulent blood serum into the ear vein. He showed on the eighth day a sharp rise of temperature and refused food. On the 14th day he had diarrhea and other symptoms of the disease, but was still strong and death would probably not have occurred for a couple of weeks. As virulent blood was required for immunizing experiments, this animal was bled to death on the 15th day after inoculation. This experiment may be considered a failure so far as the production of an acute form of hog cholera is concerned. The same procedure applied to smaller hogs would probably have been successful. as they are much more susceptible to infection.

The failure to isolate from the Hastings outbreak a hog cholera bacillus, although a number of animals were examined and the infection passed from animal to animal for several generations in the laboratory, the ease with which hog cholera bacilli can be recovered when once introduced into the system, the difficulty of infecting swine with any but large quantities of the most virulent bacilli, the readiness with which the disease is naturally spread, taken together with the results from the above filtration experiments seem to show conclusively that the hog cholera bacillus is not the primary factor in the production of hog cholera, if the disease studied were such.

Yet let us assume that the hog cholera bacillus produces in the blood of the infected animals a toxin, and that none of the toxin is used up in producing the disease. In its serial passage through hogs No. 117 and No. 124, calculating the blood as five per cent of the body weight, we would have diluted the original toxin 20,000 times. The third hog received five c.c. of this dilution or .00025 c.c. of the original, which is equivalent to .000000025 c.c. per gram weight of hog—a toxin whose potency has never been equalled except possibly in the case of purified tetanus toxin. Up to the present no substance of any great toxic

power has been obtained from bacilli of the hog cholera typhoid group, and toxins of the diphtheria tetanus type are wholly unknown among its members. In the present state of our knowledge, it seems unreasonable to suppose that a toxin of so great a potency is formed. The following experiment furnishes additional evidence that the disease-producing agent is not an unorganized toxin. Hog No. 125, weighing 20 pounds, received five c.c. of chloretone-saturated blood of hog No. 115. During the 20 days he was under observation he showed no signs of disease, and, when exposed later with an infected animal, died showing the typical lesions of hog cholera. Two-thirds of a cubic centimeter of the same blood without the addition of chloretone produced typical hog cholera when injected subcutaneously into hogs of the same size. Tri.- chlor.- tertiary- butyl-alcohol has been shown in this laboratory to be without effect upon diphtheria and tetanus toxins, but rapidly destructive to non-spore-bearing organisms. This experiment, in connection with the preceding, would seem to show that the infecting agent was some organism readily killed by mildly germicidal substances, and capable of passing through the pores of Berkefeld and Chamberland filters.

The above facts appear to place hog cholera in the group of diseases among which are rinderpest, peripneumonia,[1] and foot and mouth disease,[2] all of which are capable of being transmitted by a filterable virus. With the exception of peripneumonia, the causative organism of none of these has ever been cultivated or seen under the microscope. The size of the organism of peripneumonia which has been cultivated by Nocard and Roux lies at the limit of microscopic vision. The pathological lesions found in hog cholera are quite similar to those described for rinderpest, as seen in the Philippines,[3] and the disease is probably more closely related to this than to any of the others.

The virus differs from that of rinderpest, however, in the greater readiness with which it passes through filters[4] and its

[1] NOCARD, *Handbuch d. Path. Microorganismen*, 1903, 3, p. 682.

[2] LÖFFLER, *Centralbl. f. Bakt.*, 1898, 23, Abth. 1, p. 371.

[3] JOBLING, *Report of the Philippine Government Laboratories*, 1903, p. 363.

[4] NICOLLE and ADIL-BEY, *Ann. de l'Inst. Pasteur*, 1902, 16, p. 56.

greater resistance to physical and chemical agents. Koch[1] states that the virus of rinderpest dried at 31° C. was inert; also that the addition of 10 per cent glycerin to virulent blood destroyed its infectious qualities.

I have found that the blood serum of diseased hogs dried at 37° C. or the serum to which one-third its bulk of glycerin had been added did not suffer in virulence. When tested one month after preparation, it caused apparently as severe a form of disease as the fresh serum.

IV.

IMMUNIZATION AGAINST HOG CHOLERA.

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When preliminary experiments had shown that hog cholera could be transmitted by a filterable virus, the method employed by Kolle and Turner[2] in combating rinderpest at Kimberly, South Africa, suggested itself as applicable in this disease also. This method consisted in the simultaneous injection of virulent blood and the blood serum of an immune animal. The source of the immune blood was at first cattle that had recovered from the disease either naturally contracted or produced by inoculation with the blood of diseased animals. Later the serum of animals, subsequently hyper-immunized by the injection of large quantities of virulent blood, was used with still better success. Roger's[3] modification, the injection of a second somewhat increased quantity of infectious blood after the disappearance of the reaction from the first injection of virulent and immune blood, also seemed worthy of trial. In addition, attempts were made to immunize hogs by the injection of diseased blood dried at a temperature of 37° C. and blood to which one-third of its weight of glycerin was added.

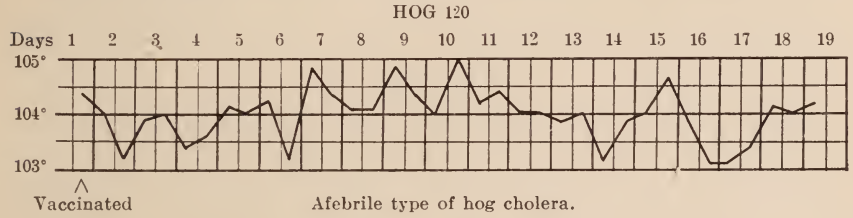
EXPERIMENT WITH DRIED BLOOD.

Hog 120, Chester-white female, 20 pounds, received subcutaneously, dissolved in normal salt solution, 0.1 gram of diseased blood which had been

[1] *Deut. med. Wchnschr.*, 1897, 23, pp. 225-41. [2] *Deut. med. Wchnschr.*, 1897, pp, 793, 818

[3] *Zeitschr. f. Hyg. u. Infectionsk.*, 1900, 35, p. 59.

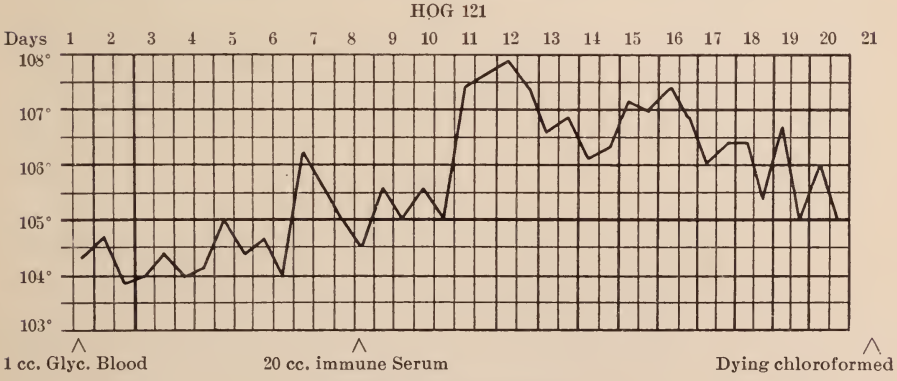
dried at 37° C. for 36 hours, 14 days previously. On the third day the animal showed loss of appetite with an inclination to remain in its litter. On the fifth day it refused food. The skin showed a bright flush, and a marked diarrhea was present. There was but a slight rise in temperature (see temperature curve). The animal gradually grew worse and died on the 29th day after inoculation. Autopsy showed typical hog cholera lesions, the colon and cecum containing numerous ulcers both of the deep and button type scattered along their length.



Afebrile type of hog cholera.

EXPERIMENTS WITH GLYCERINATED BLOOD.

I. Hog 121, male Chester-white, weighing 20 pounds, received subcutaneously one c.c. of freshly prepared glycerinated blood (blood two parts, glycerin one part). On the third day it showed loss of appetite and languor. On the fifth day refused food and was constipated. On the seventh day the temperature had risen to 105 F., and attained a maximum of 107° F. on the 12th day. The animal being moribund was chloroformed on the 20th day. Autopsy showed typical lesions, colon and cecum being covered with numerous depressed circular ulcers. The rectal mucosa was also badly ulcerated.



Febrile type of hog cholera.

II. Hog 129, Chester-white female, weighing 40 pounds, received one c.c. of same glycerinated blood now 24 days old. It showed loss of appetite on the third day, but still fed a little. On the 15th day the temperature rose suddenly, animal showed reddening of the skin, refused to eat and appeared very ill. Death occurred on the 31st day after injection. Autopsy showed typical hog cholera lesions.

These experiments showed no attenuation of the virus.

Hog 125 received five c.c. of the same diseased blood saturated with chloretone. During the 20 days it was under observation, the animal showed no signs of disease, and, when exposed with a diseased hog, contracted the disease after the regular incubation period, and died on the 22d day after exposure, showing numerous ulcers in the colon and cecum. In this instance the chloretone had apparently destroyed the infecting agent and the injection produced no immunity.

EXPERIMENTS WITH SIMULTANEOUS INJECTION OF VIRULENT AND IMMUNE BLOOD.

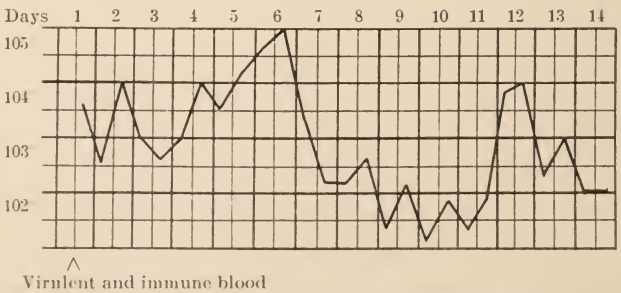
Two hogs furnished the immune serum employed. One of these, hog No. 113, was repeatedly fed infected viscera. These failed to produce any noticeable disease. The animal was subsequently further immunized by the injection of considerable quantities of filtrates. He was chloroformed and bled from the carotid. Postmortem showed no marked lesions. The colon and cecum were free from ulcers or scars.

The second hog, No. 108,* was also fed infected viscera without effect and later confined with a diseased animal, from which he contracted the disease which failed to prove fatal, but left him badly stunted. He was further immunized by the feeding and injection of large quantities of infectious material which in no instance produced any further reaction. He was chloroformed and bled from the carotid.

In both cases the blood serum was preserved by the addition of four parts per 1000 of trikresol.

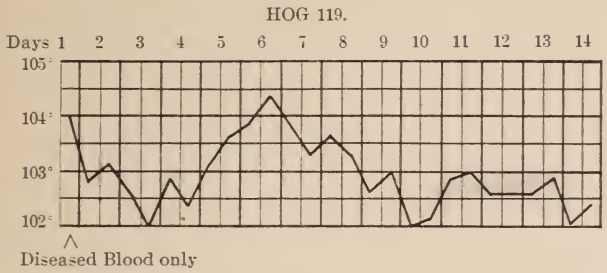
Hog No. 118, Chester-white, weight 30 pounds, received subcutaneously, on one side, one c.c. of diseased blood, and on the other side, five c.c. of immune blood. The animal showed a rise in temperature on the fourth day with loss of appetite and symptoms of hog cholera. The temperature attained its maximum on the sixth day, and fell to normal on the seventh, where it remained till the 11th day, on which a relapse set in, and the animal died on the 34th day.

HOG 118.



*This hog was fed large quantities of the viscera of two hogs that had been killed by intravenous injection of the Arkansas hog cholera bacillus. He failed to show any effects from this feeding though under observation six weeks. Later he was fed viscera from hogs naturally infected. These, however, had been kept for some months in the refrigerator, and had probably largely lost their infectious qualities.

Compare with the following:

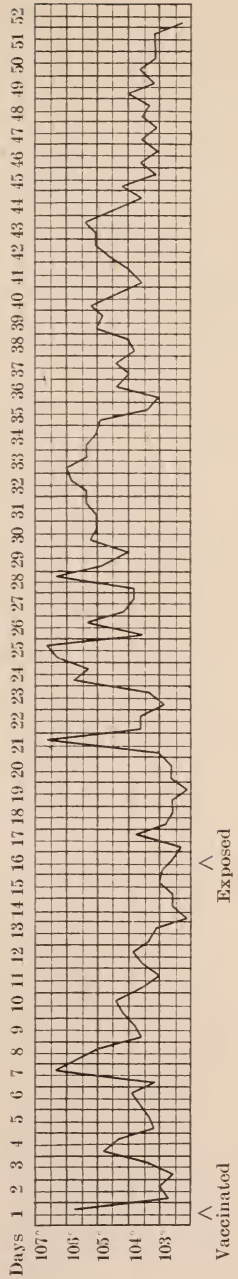


Hogs Nos. 126, 127 and 131, each weighing 20 pounds, received in the same manner as the preceding one c.c. each of diseased blood (No. 126 dried, No. 127 and No. 131 glycerinated blood) and 10 c.c. of immune blood. All showed loss of appetite and slight rise of temperature on the fourth day. The temperature attained its maximum, 106 to 107° F., on the seventh or eighth day, and in the case of 126 and 127 it returned to normal on the 11th day, and showed no further rise. In the case of No. 131 the temperature remained high until the 13th day, after which it dropped and remained normal.

On the 16th day hogs No. 126 and No. 127 were confined with a diseased hog. On the sixth day after exposure their temperature rose suddenly and a mild form of hog cholera set in. Both animals recovered; No. 127 without showing any appreciable loss of weight. In the case of No. 126, however, the disease continued longer and left the animal somewhat emaciated.

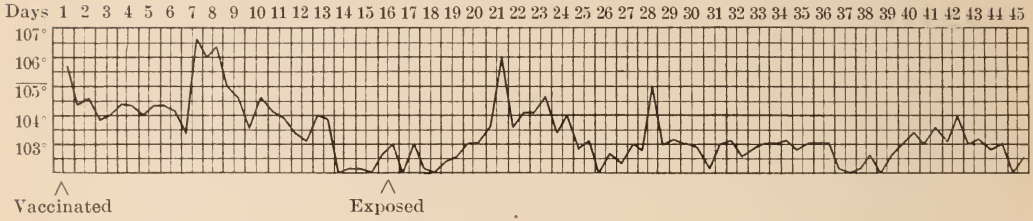
Hog No. 131, although constantly exposed with diseased animals has shown no further rise in temperature or signs of the disease.

Hog No. 130, black male, weighing 10 pounds, received subcutaneously one c.c. of glycerinated diseased blood and 10 c.c. of immune blood. It was noticed at the time of injection that the hog had a slight cough, some difficulty in breathing, and that there was a purulent discharge from the nose. He appeared quite sick on the sixth day, gradually grew worse, and died on the 18th day after inoculation. Autopsy showed typical hog cholera lesions. In addition the lungs were almost completely hepatized. This was apparently a case of latent swine plague complicating the vaccination and precipitating the fatal result.



Hogs No. 122 and 123, Chester-white sows, weighing 200 pounds each, (sow No. 122 was pregnant). Each received 20 c.c. of immune serum subcutaneously. Two days afterwards 1.5 c.c. of glycerinated blood. Five days afterward both showed loss of appetite, slight reddening of the skin and indisposition. On the 10th day they fed normally again. Twenty-one days after inoculation each received five c.c. of virulent blood. Having shown no effect from this injection 28 days afterward they received 100 c.c. of virulent blood and three and eight days later a second and third injection of 100 c.c. each, from which no effect other than a slight local reaction was observed. No. 122 gave birth at term to a litter of healthy pigs.

HOG 127.



Hogs No. 132 and No. 133, Chester-whites, weighing 100 pounds, received each, subcutaneously, 1.5 c.c. of glycerinated blood and 10 c.c. of immune serum. They became sick on the 12th and 14th day, refusing to eat and showing indisposition and a considerable rise of temperature. On the 20th day the temperature fell to normal, their appetite improved and they appeared on the 29th day, perfectly healthy. They and the sows Nos. 122 and 123 were exposed to infection from hogs in a neighboring pen during this entire period.

The results from this simultaneous method of immunization are on the whole satisfactory. The larger animals have shown themselves readily immunized against the disease, the process of immunization producing no marked illness, no considerable loss of weight, nor tendency to stunt the growth, items of the greatest importance in the practical application of any method. Younger hogs react much more strongly to the vaccination, still we have been successful in immunizing them also. The failures can be laid in the case of No. 118 to the administration of an insufficient amount of immune serum, and in the case of No. 130 to the presence of latent swine plague.

The second rise in temperature in hogs Nos. 126 and 127 may possibly be a relapse, but more likely a re-infection, the reaction from the vaccination being in both cases extremely mild and probably insufficient to produce complete immunity. This would

indicate the necessity of establishing a very nice balance between the amounts of immune and virulent blood injected in order to obtain the most perfect results.

During the entire time of immunization the animals were exposed to constant possibility of infection, which may in some degree have influenced our results.

THE CURATIVE EFFECTS OF HOG CHOLERA IMMUNE SERUM.

Hog No. 118 received on the 17th day after infection 22 c.c. of the immune serum from hog No. 113. This injection of serum had no influence upon the course of the disease, which terminated fatally.

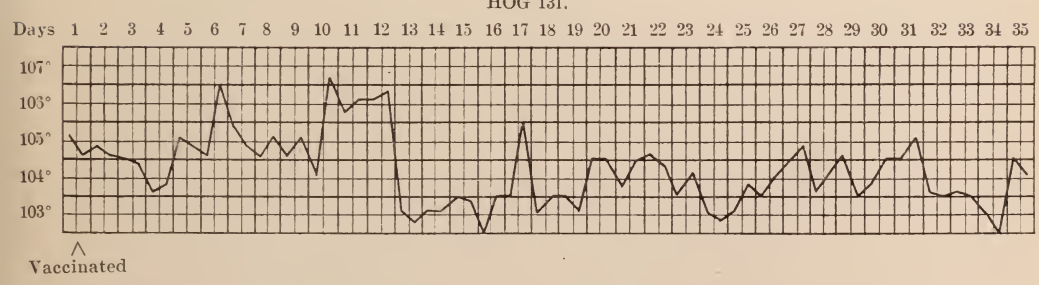
Hog No. 119 received 30 c.c. of the same serum on the 18th day after infection. This produced an immediate fall of temperature. The animal recovered: the value of the serum injection was not apparent.

Hog No. 121 received on the sixth day after infection 20 c.c. of immune serum which produced no noticeable effect either upon the temperature or upon the course of the disease which ended fatally.

Hog No. 125 received 30 c.c. on the eighth day with no effect upon the temperature or the course of the disease.

The administration of immune serum after the sixth day in the amounts given has been wholly without result. What the effect would be when given in larger quantities and earlier in the disease, we have so far made no experiments to determine.

The only record of any previous attempt to immunize hogs by a similar method to be found was that of Preiz,[1] who by the injection of the serum of a hog, that had recovered from a severe



attack of hog cholera, into swine already exposed to infection, succeeded in greatly reducing the mortality among them. We were unable to discover any further application of this idea either by Preiz or others.

[1] *Ztschr. f. Thiermedicin*, 1898, 2, p. 1.

SUMMARY

1. Hogs may be immunized against hog cholera by the simultaneous injection of diseased and immune blood, the larger animals with great readiness and apparently without danger, while the smaller animals react more violently and some loss is liable to occur.

2. Drying at 37° C. or the addition of 33 per cent glycerin does not attenuate the virus sufficiently for immunizing purposes.

3. Moderate doses of immune serum show no curative power.

The
Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. II *June 24, 1905* No. 3

THE BLOOD IN SCARLET FEVER.*

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THE absence of any exhaustive study of the blood changes occurring in measles and scarlet fever together with the suggestions offered by various incomplete reports from the literature of certain characteristic alterations in the blood of the two diseases led to an attempt on the part of the authors, in the spring of 1902, to make a systematic investigation of the subject. Such a study was made possible by the kindness of the visiting physician of one of our large contagious hospitals constantly treating a large number of the above named diseases. The report of the results obtained in the case of measles has already been published by one of us (Tileston). The present paper includes the report of the simultaneous work done on scarlatina.

LITERATURE.

As in the case of measles, a careful search yields but a small literature on the blood alterations in scarlatina. In the following summary only the salient points of the articles are given.

Many of the earlier writers report only isolated observations or results based upon faulty or inconclusive methods. Leichtenstern (1878) called attention to a moderate anemia with leucocytosis and slight diminution in the reds in scarlatina. Halla (1883) found a considerable leucocytosis, in one case 22,506 per c.mm., and a reduction in the red cells to 3,656,000 per c.mm. Later Widowitz (1887) described a rapid diminution in the percentage of

* Received for publication, Feb. 1, 1905.

hemoglobin beginning a few days after onset and a slower rise during convalescence. Pick (1890) reports one case in which he found no leucocytosis during the fever. Peé, in the same year, from the incomplete study of four cases, described an increasing leucocytosis during the early stages which disappeared with the fever.

Hayem (1889) observed a moderate leucocytosis at the beginning of the rash (10,000 to 20,000), more marked if severe angina were present, a fall of about 10 per cent in the hemoglobin and a diminution of approximately one million in the count of erythrocytes. In the case of the red cells, the lowest counts occured at the time of the fall in temperature.

The first systematic study of the blood in scarlatina was made by Kotschetkow in 1891. With respect to the blood changes, he divides the cases into three grades according to the number of white cells present, namely, 1) mild cases—10,000 to 20,000 leucocytes; 2) moderately severe cases—20,000 to 30,000 leucocytes; 3) severe or fatal cases—above 30,000 leucocytes. The whites reached 40,000 per c.mm. in several very severe cases. The increase in white cells begins from two to three days previous to the breaking out of the rash, reaches its maximum about the same length of time after its appearence and persists for a long period, finally sinking gradually to normal. Complications such as nephritis, otitis media and lymphadenitis as well as fever exert no influence on the number of leucocytes. The per cent of neutrophiles is increased in proportion to the severity of the disease. In mild or moderately severe cases the eosinophiles are normal or subnormal at the onset and rise gradually to reach their maximum during the second or third week (8 to 15 per cent); in the very severe cases they are usually diminished from the beginning and rapidly sink to zero. Contrary to the course of the neutrophiles, the lymphocytes are lessened at the onset but gradually increase during the later course of the disease. Kotchetkow believes these changes to be so constant as to be of considerable prognostic value. Beginning with the onset of the disease the number of red cells gradually sinks to about 3,000,000 per c.mm. or even lower, to rise again after the sixth week.

Rille (1892) made differential counts in three cases, two of which gave normal results. The third, terminating fatally showed a leucocytosis of 15,000 to 30,000 with a marked increase in the percentage of eosinophiles (5.31 to 7.7 per cent.)

In the study of ten cases, Rieder (1892), almost without exception, observed a considerable leucocytosis even after a prolonged period of normal temperature. Croupous pneumonia complicating one case caused an increase in the existing leucocytosis of only 1,600.

Felsenthal's (1892) results were derived from the study of six mild cases in children. The number of white cells in these varied from 18,000 to 30,000 per c. mm., and the red cells from 4,500,000 to 5,500,000. During the fever, angina and exanthem, he found a constant leucocytosis which after some weeks disappeared. The eosinophiles were increased somewhat at the time of the appearence of the exanthem (in one instance to 11 per cent), a few days later becoming less.

Zappert (1893) described an eosinophilia occuring in scarlet fever either during or just following the febrile stage. His highest count was 1,155 per

c.mm. (7.7 per cent). Studying normal children and adults, he found a higher percentage of eosinophiles in the former (four to six per cent).

Sobotka (1893) observed a leucocytosis during the incubation period of scarlet fever which persisted after the outbreak of the disease. He noted a similar leucocytosis in the incubation period of pneumonia, varicella, variola and measles.

A leucocytosis of 30,000—80,000 in oncoming scarlatina characterized at the end by a lymphocytosis was noted by Klein (1897).* Two cases complicated by nephritis, and ending in convalescence, revealed an eosinophilia of 10 to 16.5 per cent, while a third of the same type, but ending fatally, gave only one per cent.

Van den Berg's (1898) observations were made on 16 cases, 12 followed over long periods. There was a leucocytosis of 20 to 30 days duration and with a maximum value on the third to sixth day in every case. So far as he could observe this was not affected by the severity of the disease or extent of the rash. No evidence appeared in his work to demonstrate any constant relation between complications and the white count. He does not consider a prolonged leucocytosis to signify a bad prognosis. Van den Berg ascribes the high red count at the onset as due to an increase in production of cells. Postfebrile anemia was seen in seven cases (reds frequently as low as 3,500,000 per c.mm.), in one with nephritis of a severe type. The hemoglobin showed wide variations though generally high in the beginning. The differential count was as follows:—polynuclear and transitional cells 68 to 82 per cent; mononuclear cells 16 to 28 per cent; eosinophiles 1.3 to 8 per cent. In all cases these counts gradually returned to normal after the disappearance of the fever, though in a few instances the mononuclears increased to 50 per cent.

The careful studies on two adult cases of scarlet fever by Türk (1898) furnished some new data. Like the earlier writers, he speaks of a decrease in the percentage of hemoglobin and red cells, and of an increase in the number of blood plates during desquamation. He found a slight leucocytosis at first which was quickly followed by a normal count for one to two days, then a second period of leucocytosis beginning at the height of the fever or occurring with desquamation. During the first period the polynuclear cells are increased, the mononuclears diminished and the eosinophiles normal or diminished, while in the second period there is an increase in the eosinophiles and mononuclears and transitionals with a corresponding decrease in the polynuclears. Türk's white counts ranged from 8,600 to 14,000 per c.mm. The second period is, he says, often obscured by complications.

Mackie (1901) examined 25 cases at various stages and found a constant anemia (reds 3,500,000 to 4,000,000) in one-half the cases. All showed more than 10,000 leucocytes, the numbers varying with the severity of the throat symptoms, but not with the temperature. In most cases the increase in the whites began 24 hours after the appearance of the rash, reaching their maximum on the third to the tenth day, but in fatal cases the count diminished as the disease progressed.

Reckzeh's (1902) work, based upon the study of ten cases in children, is more comprehensive and thorough than any previously published. The red

*Cited by Reckzeh.

cells varied considerably during the course of the disease (3,5000,000 to 5,000,000 per c.mm.) but as a rule there was present a slight anemia. This anemia, the author thinks, is not increased by the presence of nephritis. Slight poikilocytosis was present but without polychromatophilia or granular degeneration. A corresponding moderate decrease in the hemoglobin was noted. Blood plates seemed very abundant immediately after the subsidence of the fever. The leucocytosis presents a picture more or less characteristic. Normal on the first and second day, there is a sharp rise on the third or fourth which again begins to fall on the fifth to ninth day, the count finally reaching normal at the end of the second or early in the third week. The highest count reached 41,000 per c.mm. Lymphadenitis had a marked effect in raising the leucocyte count while nephritis was without influence in two cases.

Reckzeh did not find the "second period" of leucocytosis mentioned by Türk, nor any relation between the severity of the disease and the height of the white count. The abrupt rise in the polynuclear cells, corresponding to the increased leucocyte count, took place in the first few days of the disease and was followed by a gradual sinking which reached normal or lower at the end of the first week or ten days. The lymphocytes followed a somewhat opposite course, being much diminished during the first few days then slowly increasing to become normal or slightly increased in the third week. A less definite curve was present in the case of the eosinophiles which varied from 1 to 12½ per cent. There appeared to be a more or less exact relation between the highest per cent and the fading of the rash.

Bowie (1902) though recording blood examinations in 167 cases, for the most part made only isolated observations and his conclusions are in some respects less trustworthy than would have been the case had he studied fewer cases more in detail. Two cases developed a leucocytosis two or three days before the rash broke out. The maximum count was sometimes reached during the first week and the white count became normal at any time within the first three weeks, the amount and persistence of the leucocytosis depending on the severity. All fatal cases were accompanied by a low count either from the first or later, but such a condition did not invariably indicate a fatal termination. The most favorable cases were those with a very high leucocytosis. Temperature apparently exerted no influence. Adenitis, otitis media and nephritis, according to the author, produced a rise in the number of leucocytes which preceded by a brief period their appearance. Polynuclear leucocytes, Bowie says, are much increased at first but soon fall, reaching normal about the end of the third week, while the lymphocytes take an opposite course, being at first diminished and rising gradually with the fall in the polynuclears. The eosinophiles are more variable, in the ordinary cases sinking very low at first then rising to their maximum in the first week; in the very severe or fatal cases, and with complications, they are much reduced or entirely absent. Bowie believes the blood examination to be of considerable value both in diagnosis and prognosis.

Sacquépée (1902) gives the results of his blood examinations in scarlatina in adults. His cases are divided into two groups: 1) Those with a regular course including 14 more or less grave cases, and 2) those with an irregular course, three showing complications and one ending fatally. In the first

group he found a high leucocytosis during the first three days, usually above 15,000 per c.mm., sometimes even 30,000, and after the first week rarely over 10,000. By the 20th day the number fell to 5,000 or 6,000. He was able to find no parallel between the severity of the disease and the degree of leucocytosis. During the first few days the percentage of polynuclear cells was far above the normal, often 90 per cent, but later constantly tending toward normal. The mononuclears in general followed a complementary course, the two lines finally crossing. Contrary to the course of the relative values, both types were always absolutely increased until late in convalescence when the polynuclears sank to normal. The eosinophiles at the onset, though relatively normal, were absolutely increased and by the fourth to fifth day reached even 13 per cent in some instances, remaining above normal for several weeks. The author occasionally found mononuclear eosinophiles and rarely noted the granular very small. The cases in the second group, or those with an irregular evolution, gave varying results.

Klotz, in 1904, published a most excellent paper on the leucocytosis in scarlet fever, in which he records the results of careful studies on a series of 14 cases varying from 2½ to 14 years. His division of cases is into three groups, namely: mild, medium and severe. In the first group the changes in the white cells are not striking and consist in a moderate increase in number and but a slight alteration in the proportions of the different types. In the second group, or those of moderate severity, the leucocytosis is invariably marked, as a rule 20,000 to 40,000 or even higher. The leucocytosis reaches its highest point on the third or fourth day after onset and subsequently falls gradually to normal at about the fourth to seventh week. An early increase in the polynuclear leucocytes takes place, rising to their maximnm during the first week (in some instances as much as 93 per cent) and again falling to normal by the end of the third or fourth week. The lymphocytes show an opposite course. During the first week the eosinophiles are much decreased but by the second or third week usually increase to above normal (one case to 19 per cent), again declining to normal in the fifth week. A definite relation seems to exist between the severity of the disease and the number and character of staining of the eosinophiles. In severe cases the author finds a favorable significance in a high eosinophilia and "marked oxyphile nature of the polymorphonuclear leucocytes." Certain degenerative changes in the leucocytes are mentioned. In Klotz's series complications such as lymphadenitis, arthritis, varicella and otitis showed an increase of 4,000 to 12,000 in the number of leucocytes. The effects of nephritis were variable. The author's cases of the severe or malignant type ,were associated with a low leucocyte count and low percentage of eosinophiles. Evident degenerative changes were present in the white cells.

PERSONAL OBSERVATIONS.

Our investigations include the study of the blood of 34 cases of scarlatina, 28 of whom were between the ages of 2 and 10 years, one under 2 years, and the remaining 5 varying from 11 to 23 years. Since the patients were all observed in hospital

wards, it was seldom possible to make an examination of the blood previous to the second or third day, as the patients usually did not enter until the disease was well developed. On the other hand, the examinations, once begun, were continued until death in the fatal cases, or until the establishment of complete convalescence in those ending in recovery. Only such cases were selected as gave unmistakable evidence of the disease and remained under a constant régime of diet and treatment. In no instance was any medication used, other than small doses of strychnia, and even that in but few. With almost absolute uniformity the blood was taken about 9:30 A.M. or 4 P.M., in order to avoid any possible influence by the ingestion of food.

The methods and technique employed are essentially those described by Tileston in his paper on the blood in measles recently published in this journal, and the reader is referred to his report for details. In determining the percentage values of the various types of leucocytes, the lymphocytes, large and small, mononuclears and transitional forms were counted separately. These values, however, are manifestly subject to considerable variation, depending upon the standards of the observer. Furthermore, the study of the relative values thus obtained offered no suggestions which were not equally evident from the figures obtained by combining these four groups under one head, namely, "mononuclear cells." In the tables, therefore, these are grouped under the comprehensive term "mononuclears."

CASE 1.

SCARLATINA; MEASLES DURING CONVALESCENCE.

Male. 3½ years. Entered May 3. Past history negative. Onset April 30, with fever vomiting, and "spasms." Rash appeared next day.

Physical examination, May 3: Pale child, showing considerable prostration. Mouth and throat red; papillae of tongue enlarged; tonsils enlarged, with exudate on both; eruption on hard palate; bright punctate eruption over whole body. Heart normal. Urine: albumin absent, May 4–June 1.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclear	Mononuclears	Eosins	Myelocytes	Mast Cells	Remarks
May 6	7	102.5	80	31,400	4,832,000	73.2	21.2	4	1	0.6	Rash fading. Symptoms severe, thick yellow membrane on both tonsils, culture negative. Swollen glands in neck.
" 9	10	103	75	28,000	.....	84.5	13.25	2	0	0.25	Stomatitis.
" 13	14	101	65	18,300	4,216,000	77	22	0.75	0.25	0	Slight improvement.
" 17	18	99.5	..	7,300	.....	60	35.50	3.25	0	1.25	Marked improvement; herpes labialis.
" 21	22	98.5	65	12,900	.....	58.25	36.75	3.75	0	1.25	Seropurulent discharge from ear; heart action irregular; soft systolic murmur of apex; marked desquamation.
" 25	26	100.4	60	10,500	3,370,000	75.75	19.75	3.25	0.25	1	Fever 102° yesterday.
" 28	29	101.8	..	6,800	.....	64.75	34.75	0	0	0.50	Coryza, cough, conjunctivitis. Many Koplik spots.
" 29	30	102.5	..	5,400	.....	63	35	1.75	0	0.25	Typical eruption of measles on skin and hard palate.
" 31	32	101.6	..	5,900	.....	33.25	63.75	3.0	0	0	Brilliant rash yesterday.
June 2	34	100.8	..	11,800	.....	47.25	54	1.75	0	0	Rash fading.
" 5	37	99.3	..	12,000	.....	56.25	40.75	2.25	0	0.50	
" 9	41	99.1	60	17,600	4,528,000	75	21.75	3	0	0.25	No evident cause for leucocytosis.
" 13	45	99.7	..	13,100	.....	42.25	53.25	4	0	0.50	Slight discharge from ear; sitting up.
" 18	50	98.7	65	12,300	.....	69.25	26.75	3	.25	0.75	Otorrhea continued until June 30. Discharged well July 4.

CASE 2.

SCARLATINA.

Female, 5 years. Entered May 7. Measles one year ago. Onset May 5, with fever and nausea.

Physical examination: Well developed and nourished; moderate prostration. Throat red, without exudate; papillae of tongue enlarged; rash on hard palate; bright punctate erythema over body. Soft systolic at apex. Urine: May 7–11, no albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 7	3	102	85	26,000	4,716,000	88	9.2	2.4	0.4	0	
" 10	6	99.6	80	13,900	.....	57	30.50	12	0.25	0.25	Very mild symptoms; beginning desquamation.
" 13	9	98.5	80	14,200	.....	53.25	34.25	11.75	0	0.75	Desquamation profuse.
" 17	13	98.5	80	16,100	.....	54.75	32.25	11.75	0	0.25	
" 20	16	98.7	90	12,400	.....	64	29	6.75	0	0.25	Heart irregular; soft systolic murmur.
" 25	21	99.7	85	9,000	5,056,000	51.25	42.25	6.50	0	0	Desquamation continues.
June 1	28	99	80	19,700	.....	60	29.75	9	0.25	1	Severe eczema about and inside nose; culture negative.
" 5	32	98.9	..	19,100	.....	53.75	36	6.25	3.75	0.25	Eczema nearly healed.
" 9	36	98.7	..	14,100	.....	....	....	....	....	....	
" 14	41	....	80	18,800	.....	65	30.75	3.25	0	0	Still desquamating.
" 18	45	....	85	15,000	.....	61	32	7	0	0	Nasal discharge ceased.
" 26	53	....	..	12,000	.....	52	41.25	6	0	0.75	Culture negative; discharged well.

CASE 3.

SCARLATINA.

Female, 2½ years. Entered May 8. Past history negative. Present illness began May 7 with vomiting, fever, and rash.

Physical examination: Well developed and nourished; but little prostration. Throat red; exudate on both tonsils; papillae enlarged. Brilliant punctate eruption on body and roof of mouth, typical of scarlatina. Heart normal. Urine: May 9, no albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mono-nuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 9	3	100	100	44,000	4,900,000	89	9.4	1.4	0.2	0	Brilliant rash.
" 12	6	98.8	85	21,000	.....	62.2	31.6	6	0	0.2	Mild course; rash fading.
" 15	9	98.6	85	25,000	.....	63	34	2	0	1	Desquamation began on 13th.
" 18	12	98.8	75	19,400	4,896,000	64	30.75	5	0	0.25	Desquamation general.
" 21	15	98.5	..	23,700	.....	55.25	41.75	3	0	0	Doing well.
" 25	19	98.8	80	22,100	.....	66	28	5	0.25	0.75	
" 30	24	....	75	23,900	.....	48.75	26.75	0.25	0	0.25	Small "run-around" on thumb of five days' duration; a second on the 31st.
June 5	30	....	..	34,000	.....	48.75	44.25	6.25	0	0.75	Paronychia improving.
" 11	36	....	..	79,500	.....	22.75	75.00	2.25	0	0	Nothing to account for leucocytosis.
" 18	43	....	75	46,900	.....	31	60.75	4.75	3.25	0.25	Spleen palpable; liver and lymph nodes not enlarged.
" 24	49	....	85	21,600	5,292,000	46.75	51.25	1.75	0	0.25	Discharged well, June 26.

CASE 4.

RELAPSE AFTER SCARLATINA.

Boy, 7 years. Entered March 25. Measles last year; pertussis in January, 1902. Entered March 25 for diphtheria which began March 16 with vomiting, fever and sore throat. Four days later noted that tongue was very red, and the papillae enlarged.

Present illness: April 21 transferred to scarlatina pavilion, with typical desquamation of scarlatina on trunk, legs and feet. May 9, temperature 101. May 10, bright punctate eruption on skin and roof of mouth, papillae red and swollen; throat red. Systolic murmur at apex. Temperature 100. No vomiting. Urine: April 21 and 28, May 21 and 26: No albumin. Profuse desquamation.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mono-nuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 11	3	100.2	65	21,000	5,112,000	80.25	11	8.75	0	0	Brilliant rash; slight prostration.
" 14	6	99.5	70	16,000	.....	57.25	39.25	3.50	0	0	Euphoria; rash nearly gone.
" 17	9	98.3	70	18,600	.....	59.75	32.25	7	0	1	Rash gone; throat clear.
" 20	12	98.4	80	12,600	5,452,000	63.25	28.75	6.75	0	1.25	Fresh desquamation due to recrudescence.
" 25	17	98.6	..	19,400	.....	78.75	19.75	0	0.25	1.25	Vomiting for past two days; throat and urine negative.
" 28	20	98.2	..	11,700	.....	45.25	52.75	1	0	1	Occasional vomiting; desquamation general.
June 1	24	.....	75	19,600	.....	61	33.75	5.25	0	0	Still desquamating.
" 4	27	.....	..	10,300	.....	18	74.75	6.25	0	1	
" 9	32	.....	..	16,700	.....	69	22	6.25	0.25	2.50	Desquamation nearly gone.
" 12	35	.. ...	..	18,500	.....	67.25	55	6.75	0	1	
" 17	40	.....	..	17,300	.....	54.75	39.75	4	0	1.75	Desquamation complete.
" 24	47	.....	70	13,300	.....	50.75	44.25	4	0.25	.75	
" 29	52	.....	..	11,600	.....	49.75	40	5	5.75	0	Discharged well June 30.

CASE 5.

SCARLATINA; ENDOCARDITIS; DIPHTHERIA.

A sailor, 18 years. Entered May 12, 1902. Measles last summer. Present illness began May 11, with diarrhea, headache and sore throat; fever and rash noticed on following day, the latter first appearing on chest.

Physical examination, May 12: Well developed and nourished; marked prostration Throat red; tongue thickly coated with enlarged papillae; tonsils swollen with exudate, which also covers the posterior pharyngeal wall; brilliant, punctate eruption on body and roof of mouth. Loud systolic murmur at apex; cardiac dullness normal. Temperature 104°. Urine, May 13: trace albumin, no casts; June 5, very slight trace; June 21, none.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 12	2	104	100	17,200	5,208,000	95.25	4.75	0	0	0	
" 14	4	103.4	..	13,600	.....	93.25	5.25	.50	1	0	Very sick; brilliant rash, confluent on thighs.
" 16	6	102.2	90	7,500	.....	79.25	19.75	1	0	0	Rash persists; throat and mouth in bad condition; delirium.
" 18	8	102.1	90	13,200	.....	84.75	14.75	.25	0	0.25	Purulent discharge from nose; membranes coughed up; no diphtheria bacilli but many streptococci in culture.
" 20	10	101.8	..	11,800	.....	.....	.....	....	....	....	Stupor; yellowish membrane on left tonsil.
" 22	12	103.3	95	14,400	4,525,000	81	17.75	.25	0	1	Heart enlarged; loud systolic murmur at apex.
" 25	15	103.1	..	10,100	.....	81	17.75	1.25	0	0	Otitis media purulenta; diphtheria bacilli in throat culture.
" 28	18	98.6	..	.....	.....	63	34.75	1.25	0.25	.75	Temperature normal; a general improvement; profuse desquamation.
" 30	20	98.5	75	13,300	.....	64.25	33.75	.25	0	1 75	Heart same; bacilli still present.
June 5	26	98.2	..	10,300	.....	66.75	31.75	.25	0	1.25	Cardiac dullness diminished; sounds of better quality; marked improvement.
" 13	34	98.6	80	7,600	4,448,000	72.75	23.75	2.25	0	1.25	Heart much enlarged; loud blowing systolic murmur at apex and in axilla; accentuated pulmonary second sound. Discharged July 18.

CASE 6.

SCARLATINA.

Girl, 7 years. Entered May 14. Diphtheria, varicella, and pertussis previously. Present illness began May 11, with vomiting, fever and angina.

Physical examination, May 14: Well developed and nourished; moderate prostration. Throat red with some exudate on tonsils; papillae much increased in size; punctate erythema on roof of mouth and over body, emphasized in groins and axillae. Slight systolic murmur. Urine: no albumin. Temperature 103.5°.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 15	5	101	80	16,900	4,932,000	62	12	.26	0	0	Very mild case; bright rash.
" 17	7	98.7	..	12,600	.....	35.75	61.75	1.25	.25	1	Rash nearly gone; beginning desquamation.
" 20	10	98	..	13,100	.....	49.5	47	3.5	0	0	General desquamation.
" 25	15	100	..	23,400	.....	67.25	26.25	6.50	0	0	Convalescence.
" 28	18	98.8	..	15,500	.....	65.50	29.25	3.25	0	2	Herpes labialis.
" 31	21	98.5	80	16,300	.....	66.25	29.75	3.75	.25	..	
June 5	26	.....	..	8,900	.....	37	53.75	6	2.25	1	Paronychia on two fingers; vaccination June 6.
" 10	31	.....	..	10,700	.....	76	20	3.50	0	.50	Still some desquamation.
" 12	33	103.5	..	8,900	.....	54.25	43.50	2	.25	..	Temperature probably due to vaccination.
" 18	39	.....	70	9,800	5,044,000	24	62	12	0	2	Discharged well, July 21.

CASE 7.

SCARLATINA; DIPHTHERIA.

Girl, 4 years. Entered May 17. Diphtheria two years ago. Present illness began May 15, with sore throat and vomiting; rash appeared on the 17th.

Physical examination: Well developed and nourished; general condition good, "strawberry" tongue; throat red; exudate at mouths of crypts on tonsils; faint punctate erythema on roof of mouth, abdomen and extremities, emphasized in axillae and groins. Enlarged lymph nodes in right side of neck. Paronychia of some duration on finger, with loss of nail; superficial impetiginous lesion of foot. Heart normal. Urine: May 15, no albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 17	3	101	80	21,000	4,508,000	88.50	11.25	0	0	0.25	
" 19	5	.....	..	.....	.....	82.25	14	2.75	1	0	Rash bright on thighs, fading elsewhere.
" 20	6	100.8	60	25,900	.....	62	31.50	6.25	0	0.25	Beginning desquamation.
" 23	9	100	..	15,400	.....	60	40	0	0	0	Patient sitting up.
" 27	13	99.6	60	12,800	4,204,000	69.75	28.25	2	0	0	
" 31	17	98.4	..	9,100	.....	70.75	27.25	1.50	0.25	0.25	
June 7	24	99.2	..	13,200	.....	53.50	46.25	0	0	0.25	Patient about the ward.
" 16	33	98.5	70	12,100	4,868,000	58	37	5	0	0	
" 22	39	102.9	..	.....	.....	.....	.....	....	....	....	Membrane on tonsils; culture shows diphtheria bacilli.
" 28	45	98	..	21,600	.....	84	16	0	0	0	Throat clear on 24th; antitoxin urticaria. Discharged well July 11.

CASE 8.

SCARLATINA.

Girl, 2½ years. Entered hospital May 16. Past history negative. Present illness: Onset, May 14, with sore throat and vomiting.

Physical examination: Rachitic rosary; considerable prostration. Throat and tongue red, papillae much enlarged; bright red punctate erythema on roof of mouth and thickly over body, especially in axillae and groins. Systolic murmur at apex. Temperature 103.2°. Urine: May 17, slight trace albumin; May 24, slightest possible trace; June 11, trace albumin; hyaline and granular casts, normal blood and renal cells.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 18	5	101.3	65	16,600	3,749,000	54.25	39.25	6.50	0	0	Rash still bright.
" 20	7	100.8	..	28,300	.....	60	36	2.75	0.25	1	Rash fading; desquamation beginning on neck and arms; cervical lymph nodes swollen.
" 21	8	100.8	..	34,600	.....	75	23	1.75	0.25	0	Patient improving.
" 23	10	100	..	25,800	.....	70	25	1.50	0.50	0	Typical desquamation; lips swollen and bleeding.
" 27	14	98.8	65	20,300	4,412,000	67.25	29	3.50	0	0.25	
" 31	18	98.7	60	15,200	.....	....	....	....	....	....	
June 6	24	102.2	..	26,400	.....	62.75	35.75	0.75	0.25	0.50	Intermittent fever of unknown cause.
" 14	28	101	..	21,600	.....	72.50	24.50	1	1.75	0.25	Double otitis media with profuse purulent discharge; cervical adenitis.
" 22	36	100	55	11,200	4,744,000	53	43.50	1.25	1	1.25	Paronychia.
" 29	43	....	65	11,400	.....	41.75	57.75	0.50	0	0	Grad. improvement; aural discharges ceased. Discharged well July 25.

CASE 9.

SCARLATINA, NEPHRITIS, DIPHTHERIA.

Boy, 5 years. Entered May 19. Past history: measles and pertussis. Present illness Onset, May 17, with vomiting, fever and rash.

Physical examination: Well developed and nourished; only moderate prostration. Mouth and throat red; papillae hypertrophied; no exudate; fading punctate erythema on chest, abdomen and thighs, also on hard palate. Heart normal. Temperature 101.3°. Urine: May 20, no albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 19	3	101.3	85	23,100	4,720,000	....	....	....	....	....	
" 21	5	100	..	18,100	.....	72	23.25	3.75	0.25	0.75	Profuse rash on thighs, fading elsewhere.
" 23	7	99.5	80	21,500	.....	67.75	27.25	3.25	1.75	0	Beginning desquamation; euphoria; systolic murmur at apex.
" 27	11	98.8	..	16,800	.....	66.25	31.25	1.75	0.75	0	Typical desquamation.
" 30	14	100	..	27,300	.....	75.25	30	3.50	0.75	0.50	
June 3	18	100.8	..	17,500	.....	67.50	29	3.25	0	0.25	Cervical lymph nodes much enlarged.
" 8	23	98.8	..	20,800	.....	72.50	26.25	1.25	0	0	Face puffy; vomiting; urine of acute nephritis.
" 11	26	98.8	..	25,900	.....	74.25	21.25	3.75	0	0.75	Marked symptoms nephritis; urine as on 8th.
" 14	29	98.7	55	22,500	4,506,000	68	27.25	4	0.25	0.50	Some improvement.
" 18	33	98.6	..	19,600	.....	58.25	37	4.25	0	0.50	Systolic murmur at apex.
" 23	38	98.4	60	19,400	.....	59	39	2	0	0	Gaining steadily; only trace albumin.
" 27	42	....	60	17,800	4,515,000	41	55	3.25	0.25	0.50	
July 2	47	....	..	13,900	.....	44.50	50.50	5	0	0	
" 7	52	....	60	12,100	.....	46.75	49.75	3.25	0.25	0	Patient up.
" 14	59	101.7	50	16,100	.....	60	39.25	0.75	0	0	Slight relapse of nephritis.
" 17	62	99	60	10,600	4,632,000	56	42	3	0	0	
" 19	64	103.1	..	14,500	.....	58.25	39.75	1.50	0.50	0	Membrane on left tonsil; diphtheria bacilli present.
" 22	67	98.5	..	13,700	.....	63.50	34	2	0.50	0	
" 26	72	....	..	12,800	.....	52	46.75	0.75	0.25	0.25	Still some symptoms of nephritis; throat clean. Discharged Aug. 2.

CASE 10.

SCARLATINA, DIPHTHERIA.

Colored girl of 6 years. Entered May 19. No history obtainable.

Physical examination: Well developed and nourished; does not appear sick. Throat negative; tonsils slightly swollen; papillae red and prominent; no skin eruption; typical flaky desquamation on knees, elbows and hands, finer on trunk. Judging from the desquamation, the disease is of about two weeks duration. Heart irregular, not enlarged; soft systolic murmur at apex; pulmonic second sound slightly accentuated; occasional sibilant râle. Temperature 98.5°. Urine: May 19, no albumin; May 24 and June 24, slightest possible trace.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 19	14	98.5	..	13,400	.....	44	54.25	1.75	0.75	0.25	
" 22	17	98.3	..	20,200	.....	50.25	47.75	2	0	0	Convalescence; slight bronchitis.
" 25	20	98.5	..	20,400	.....	50.75	48	0.75	0.25	0.25	
" 28	23	....	70	13,600	4,824,000	60	26.25	13.75	0	0	
June 1	27	....	..	14,000	.....	60.50	37.50	1.75	0	0.25	Severe bronchitis.
" 6	32	....	..	7,600	.....	47	50.50	2.50	0	0	Cough improving.
" 10	36	103.3	..	.....	... ..	....	....	....	....	....	Sore throat; membrane on tonsils; cervical nodes enlarged.
" 12	38	98.5	50	15,200	.....	56.50	40.75	1.75	0	1	Diphtheria bacilli found in nasal secretions and throat.
" 16	42	98.5	60	16,700	4,200,000	65.75	33.25	1	0	0	Throat clean; desquamation.
" 21	47	103	65	9,400	.....	67.25	29.75	2	0	1	Antitoxin urticaria; temperature normal on the 23d.
" 28	54	....	50	12,900	3,818,000	61.50	36	1.50	0	1	A few râles; systolic murmur probably functional. Discharged well July 2.

CASE 11.

SCARLATINA.

Colored boy of 3 years. Entered May 20, 1902. Measles six months ago. Present illness: Onset, May 19, with fever and sore throat.

Physical examination: Well developed and nourished; condition good. Fauces red, without exudate; papillae of tongue enlarged and red; punctate eruption on roof of mouth; discrete eruption over whole body, the swollen mouths of the hair follicles showing up plainly on the black skin. Heart negative; a few coarse râles. Urine: No albumin on repeated examination.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 20	2	100.4	..	11,800	.....	83	14.75	2.25	0	0	
" 22	4	99.5	75	12,100	4,396,000	79.50	17.50	2.50	0	0.50	Mild Case.
" 25	7	101.5	..	19,700	.....	80.25	18	1.75	0	0	Rash fading.
" 28	10	101.3	..	18,100	.....	70	27.50	2.25	0	0.25	Desquamation.
" 31	13	98.2	85	16,500	.....	68.75	26.25	2	0	1	Doing well.
June 3	16	100	..	9,300	.....	....	....	....	....	....	
" 8	21	79.2	..	12,500	.....	54	42.50	1.50	0.75	1.25	Good convalescence.
" 17	30	98.5	60	8,400	5,292,000	61.75	34	3.50	0	0.75	Discharged well July 10.

CASE 12.

SCARLATINA.

Boy, 7 years. Entered May 21. Past history: Measles and diphtheria previously. Present illness began May 7, with rash.

Physical examination: Well developed and nourished. Glands in neck much enlarged; throat negative; papillae swollen; no eruption; skin rough with "breaks" at fingers and toes. Slight systolic murmur at apex. Temperature 99.4°. Urine: no albumin May 22; May 30, slightest possible trace; June 4, slight trace with few granular and epithelial casts, some with blood adherent; small and large round cells. June 16, only slightest possible trace albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 21	15	99.4	..	16,200	.....	78.50	17.75	3	0.25	0.50	
" 23	17	99.8	60	13,100	4,124,000	52	38.50	7.50	0	2.00	Slight desquamation.
" 27	21	98.8	..	12,600	.....	46.50	45.50	7.25	0	0.75	Inconstant systolic murmur at apex with accentuation of pulmonary second sound; no increase in area cardiac dullness.
June 1	26	98.6	..	13,700	.....	54.25	41.75	4	0	0	
" 3	28	104.2	..	35,500	.....	87.50	10.75	0	1	0.75	Temperature probably due to adenitis.
" 6	31	98.3	..	11,600	.....	61.25	33.50	5	0	0.25	
" 10	35	100	..	15,800	.....	40.50	53	6	0	0.50	
" 15	41	99.4	60	11,300	4,676,000	68	25	5.50	0.25	0.25	
" 22	47	100	60	12,800	.....	43.25	49.75	6	0.25	0.75	Alveolar abscess. Discharged well, July 11.

CASE 13.

SCARLATINA.

Boy, 2½ years. Past history: Measles. Present illness: began May 20, with rash.

Physical examination, May 21: Well developed and nourished, only little prostration. Slight nasal discharge; papillae of tongue enlarged and red; throat somewhat reddened; no rash on roof of mouth; punctate erythema all over body; bright on thighs and lower abdomen, fading elsewhere. Soft systolic murmur at apex; heart otherwise normal. Temperature 99.9°. Urine: no albumin May 23 and 24.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 21	2	99.9	..	12,200	.....	75.25	20.75	4	0	0	
" 24	5	101.7	65	15,000	4,580,000	45.50	53.50	0.75	0	0.25	Mild course, temperature normal on the 25th.
" 27	8	98	..	11,500	.....	67	32	1	0	0	
" 30	11	98	80	14,200	.....	52.50	45.25	1.75	0	0.50	Doing well.
June 5	17	....	..	11,700	.....	50	46	4	0	0	Typical desquamation on hands; slight discharge from ear.
" 11	23	....	..	16,400	.....	64	35.25	0.50	0	0.25	Vaccination.
" 14	26	98.7	80	11,200	.....	63.75	35	0.25	0.75	0.25	Temperature 103.3° on the 12th, due to blocking up of pus in ear.
" 18	30	100	..	14,500	.....	55.50	42	2.50	0	0	Reaction from vaccination.
" 27	39	99	85	14,600	4,936,000	58.25	39	2.75	0	0	Considerable inflammation at seat of vaccination Discharged well. July 14.

CASE 14.

SCARLATINA.

Girl, 13 years. Measles at age of 5, pertussis at 8. Present illness: Onset May 19 with slight sore throat, fever and headache accompanied by an eruption on the body. Only mild symptoms: no prostration.

Physical examination May 23: Well developed and nourished. Throat red; no exudate; papillae of tongue enlarged; enlarged lymph nodes in neck: punctate erythema on roof of mouth, trunk and extremities. Soft systolic murmur at apex, not transmitted; heart otherwise normal. Urine: May 23 and July 5, no albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 23	5	99.6	80	12,200	5,088,000	65.75	23	11.25	0	0	
" 25	7	98.7	..	12,100	.....	46.75	43.25	9	1	0	Rash gone; beginning desquamation.
" 27	9	98.3	..	14,300	.....	50.50	43	6.25	0	0.25	Very mild case; pronounced desquamation.
" 29	11	98.4	..	13,200	.....	42.50	47.25	9.75	0	0.50	
June 1	14	98.4	80	16,000	.....	62.25	33	3.75	1	0	Typical desquamation.
" 3	16	98.8	..	20,900	.....	40.50	52	5	0	2.50	Convalescence.
" 6	19	98.5	..	18,600	.....	48	46.25	4	0.75	1	Up on the 8th.
" 10	23	....	..	11,800	.....	49	45.25	5.75	0	0	
" 14	27	....	80	10,800	.....	48.50	44.25	6.50	0	0.75	
" 19	32	....	80	15,300	5,364,000	39.25	50.75	9	0	1	Nothing objective to account for leucocytosis.
" 23	36	....	..	18,800	.....	61	28.50	9.25	0	1.25	
" 27	40	....	..	13,800	.....	52.75	39.75	6	0	1.50	
July 1	44	....	80	16,400	.....	60	34	5.75	0.25	0	
" 5	48	....	..	11,800	.....	40.50	54	4.50	0	1	
" 9	52	....	85	11,400	.....	37.25	52.25	9	0	1.50	Discharged well, July 12.

CASE 15.

SCARLATINA.

Girl, 10 years. Past history: varicella, pertussis and measles. Present illness: Onset May 25, with sore throat, vomiting, headache and rash.

Physical examination May 27: Well developed child; much prostration; tender, enlarged lymph nodes in both sides of neck; throat red, with exudate on tonsils; papillae enlarged and red; brilliant punctate eruption on roof of mouth and well over body, with much erythema. Soft systolic murmur, pulmonary second sound accentuated; heart not enlarged. Urine: May 28 and June 2, no albumin. Temperature, 103°.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 27	3	103	..	15,800	.....	75.75	24.25	0	0	0	
" 29	5	102.7	..	13,600	.....	57.50	41.50	0.25	0.75	0	Very sick; brilliant rash.
" 31	7	100	..	17,200	.....	57	36	6.25	0.75	0	Improving.
June 3	10	98.4	..	16,300	.....	57	40.50	1.50	1	0	Eruption fading on the 1st
" 6	13	98.5	..	13,600	.....	86.50	12.50	1	0	0	Typical desquamation; deafness.
" 9	16	98.5	..	15,200	.....	70.75	27.25	0.50	0.50	0	Profuse desquamation; no otitis media.
" 13	20	....	..	10,900	.....	50	46	2.50	0.50	1	General condition good.
" 18	25	....	65	17,000	4,160,000	60	35.25	3.50	1	0.25	
" 22	29	....	60	13,500	.....	46	51.75	1	0	1.25	Feet and hands still desquamating.
" 26	33	....	..	17,400	.....	60	36	3.25	0	0.75	Up about ward.
" 29	36	....	65	15,400	4,128,000	55	42.25	2.75	0	0	Desquamation complete, except feet.
July 3	40	....	..	14,400	.....	51	47.25	2.75	0	0	
" 8	45	....	65	14,200	4,214,000	70	26.75	2	0	1.25	
" 15	52	....	60	15,300	4,486,000	59	33.50	6.75	0.50	0.25	Discharged well, July 18.

CASE 16.

SCARLATINA WITH PERITONSILLAR ABSCESS.

Woman, 20 years old. Measles six years ago. Present illness began May 28, with sore throat, headache, vomiting, chill, fever and general prostration.

Physical examination, May 29. Well developed and nourished; considerable prostration. Coryza; throat red; tonsils swollen, with exudate; papillae of the tongue enlarged; punctate eruption on roof of mouth; enlarged tender lymph nodes of neck. Typical punctate erythema over body. Heart enlarged 1 cm. to left of nipple line; loud systolic murmur heard all over the precordia, in the axilla and back. Pulmonic second sound not accentuated. Urine: no albumin. Throat cultures negative, May 28.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 29	2	101.7	..	30,000	.....	96.25	3.75	0	0	0	Bulging in front left tonsil
" 31	4	100	..	18,700	.....	95.25	4.75	0	0	0	Copious discharge pus from abscess of tonsil.
June 3	7	98.5	..	14,000	.....	73	23.50	3	0	.50	
" 6	10	98.5	..	15,200	.....	57	40.50	.50	1	1	Condition good.
" 9	13	98.5	..	14,600	.....	83.50	15.50	1	0	0	
" 14	18	....	..	9,800	.....	67.25	24	8	.75	0	Typical desquamating.
" 19	23	....	85	10,200	5,395,000	.. ..	.....	....	....	....	Up
" 26	30	....	80	11,300	.....	57.50	40	.50	1	1	Feet still desquamating.

CASE 17.

SCARLATINA.

Girl, 3 years old. Present illness began May 28, with sore throat and fever.

Physical examination, May 29. Moderate prostration; considerable nasal discharge; throat red; exudate on tonsils; papillae of tongue enlarged; typical punctate erythema over body. Systolic murmur at apex. Enlarged tender cervical lymph nodes. Urine: no albumin, May 30. Cultures from throat and nose negative, May 29 and 30, June 5, 21 and 22.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 30	3	101.4	70	31,800	4,908,000	72	28	0	0	0	Rash bright on legs, fading elsewhere; thick yellow exudate on tonsils.
June 1	5	101.4	..	32,300	.....	73.25	26.25	0.50	0	0	Throat culture negative; examination throat negative; antitoxin 8,000 units.
" 4	8	99	..	29,200	.....	60	36	1.75	1.75	0.75	Throat improving.
							22.50	2	1	0	Throat and tonsils free from exudate.
" 7	11	98.5	..	47,200	.....	74.50					
" 11	15	98.4	..	26,500	.....	75.50	24	0.50	0	0	Desquamation; throat negative.
" 15	19	98.2	..	9,300	.....	42.75	55.25	2	0	0	Antitoxin rash.
" 21	25	98.7	65	24,600	4,348,000	51.25	44.25	4.50	0	0	Doing well; Temperature 101.4 on the 16th.
" 26	30	98.2	65	24,600	.....	58.50	37.50	3	1	0	Still desquamating.
July 3	37	....	60	17,000	.....	40.75	57.25	1	0	1	
" 10	44	....	65	17,600	.....	35.75	63.75	0.50	0	0	Irregular heart; systolic murmur at apex.
" 18	52	....	75	19,500	5,027,000	41	56.75	1.75	0	0.50	
" 25	59	....	..	19,400	.....	42	55	1.25	0	1.75	Discharged well, July 28.

CASE 18.

SCARLATINA.

Boy, 3½ years. Entered May 29. Present illness: Pertussis for some weeks, still whoops.

Physical examination: Robust child; much prostration. Profuse coryza; throat red but no exudate; strawberry tongue; rash on roof of mouth; brilliant punctate erythema all over body; brightest on legs, fading on trunk. Systolic murmur at apex; heart not enlarged. A few fine moist râles (pertussis). Whoops occasionally.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mono-nuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 30	9	102.2	..	28,900	.....	66	33.50	0.50	0	0	
June 1	11	100.8	..	31,900	.....	77.75	19.	1	2.25	0	
" 4	14	102	..	27,500	.....	67.	30.25	2	0	0.75	Considerable nasal discharge.
" 7	17	98.8	..	26,700	.....	74.50	23.50	1.75	0	0.25	Desquamation beginning; throat negative; vaccination on the 6th.
" 16	26	98.5	..	12,700	.....	60.25	37.25	1.50	0	1	Typical desquamation; cervical lymph nodes enlarged.
" 22	32	....	60	21,700	4,008,000	50	44.25	3.50	2	0.25	Nothing to account for leucocytosis.
" 25	35	....	..	16,900	.....	.....	.....	...	....	....	
" 29	39	....	55	17,000	.....	52.25	45.25	2.25	0	0.25	
July 8	48	....	65	15,200	.....	54.50	44.50	1	0	0	
" 14	54	....	65	11,900	.....	65	34	1	0	0	Discharged well July 19.

CASE 19.

SCARLATINA.

Boy, 7 years. On May 22 is said to have had macular eruption on hands and wrists; preceded by cough and sore eyes for several days (measles?). On May 28, scarlet rash came out on body.

Physical examination, May 30: Vigorous; moderate prostration. Eyes suffused, conjunctivae congested; throat red but no exudate; papillae of tongue swollen; punctate eruption on roof of mouth; on trunk, legs and arms, fading punctate rash characteristic of scarlatina; branny desquamation on face. Systolic murmur at apex; no râles. Temperature 103°. Urine: No albumin June 2, 14 and 23.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mono-nuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 30	3	100	..	11,400	.....	58	38.75	3.25	0	0	
June 1	5	98.7	..	15,200	.....	57.25	39.25	2.50	0.25	0.75	Temperature 104° June 2; enlarged tender lymph nodes in neck.
" 4	8	101.1	..	20,600	.....	77.75	20.25	0.75	0	1.25	
" 7	11	98.9	..	25,700	.....	75.50	24.50	0	0	0	Discharge from right ear.
" 10	14	98.6	..	12,100	.....	61.50	35	2.50	0	1	Faint systolic murmer at apex.
" 16	20	99.5	..	13,100	.....	.....	.....	....	....	....	Fresh swelling of glands of neck; right ear still discharging.
" 22	26	102.7	..	15,000	.....	70.25	25.25	4	0	0.50	Temperature apparently due to enlarged lymph nodes.
" 27	31	98.5	65	12,200	4,148,000	61	31	7.75	0	0.25	
July 7	41	98.8	60	12,600	4,797,000	54.25	40	2.50	0	3.25	Discharged well July 22.

CASE 20.

SCARLATINA WITH NEPHRITIS.

Boy, 5 years. Two sisters ill with scarlatina. Present illness began May 10, with rash.

Physical examinations May 30: Healthy child; pronounced desquamation, typical of scarlatina, on hands and legs, slightly on back; papillae of tongue enlarged; chronic enlargement of tonsils; enlarged lymph nodes behind the left ear. Heart slightly irregular, sounds normal. No edema. Temperature 101.1°. Urine: May 31; large trace of albumin; smoky; contains casts and blood.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclear	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 30	21	101.1	..	23,100	.....	78.25	19.75	1	0	1	
June 1	23	99	..	16,800	.....	63.50	34.50	2	0	0	
" 5	27	101	..	14,900	.....	73.	25.50	.50	0	1	Trace of albumin in urine.
" 8	30	99	..	9,600	.....	52.75	43.50	1	1.75	1	Lymph nodes in neck enlarged; systolic murmur over precordia.
" 11	33	98	..	12,400	.....	50.50	48	1.25	0	.25	Trace albumin in urine.
" 17	39	98.8	55	16,200	.....	52.25	43.25	4.50	0	0	Still desquamating.
" 21	43	98.5	..	22,700	.....	87.	12.	.25	0	.75	Temperature 105° last night, probably due to enlarged glands in neck.
" 24	46	99	55	13,100	3,603,000	51.25	46.75	2	0	0	Marked improvement.
" 27	49	102.5	..	18,500	.....	77.75	22	0	0	.25	General condition better, but glands in neck again enlarged.
July 2	54	98.8	..	9,300	.....	52.50	44.50	2.25	0	.75	Some achromia and poikilocytosis; no nucleated red cells.
" 6	58	....	55	10,400	3,811,000	40.50	53.50	5	0	1	Discharged well July 8.

CASE 21.

SCARLATINA, BRONCHOPNEUMONIA.

Boy, 1¼ years. Measles six months ago. Present illness began May 20 with sore throat, fever and vomiting. Rash appeared three days later. History of exposure to scarlatina.

Physical examination May 30: Strong appearing child; extreme prostration. Cyanosis; some retraction of lower ribs with respiration; tracheal râles; large tender lymph nodes on both sides of neck; profuse nasal discharge; pharynx red with exudate on posterior wall; tonsils clear; papillae on tongue enlarged; no rash; no desquamation. Heart action weak; systolic murmur. Lungs full of râles, with patches of bronchial breathing in right axilla and back. Spleen palpable. Cultures of throat negative. Temperature 102°. Antitoxin, 8000 units.

Blood examination, May 30 (11th day of disease), Hemoglobin 65%; Leucocytes 23,900 per c. mm.; differential count—polynuclears 73.25%; mononuclear 26%; eosinophiles .5%; myelocytes .25%; slight achromia and poikilocytosis.

No improvement with stimulation, death at 4 P. M., May 31. No autopsy.

Examination on 31st showed dullness with bronchial breathing below the angle of the scapula on the right and patches of bronchial breathing in the left lung.

CASE 22.

SCARLATINA.

Boy, 5 years. Measles four years ago, pertussis a year later. Present illness: Onset June 2, with headache, cough and fever.

Physical examination, June 6: Well developed boy; moderate prostration; nasal discharge; throat red with membrane on tonsils and uvula; papillae of tongue red and swollen; punctate erthyema on roof of mouth and brilliant over body; cervical lymph nodes enlarged. Systolic murmur at apex. Temperature 103.8°. Urine: No albumin June 6 and 13.

Date	Day Disease	Temperature	Hemaglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclear	Mono-nuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 7	6	99.3	..	27,800	.....	65	28	6	.75	.25	Severe adenitis; throat culture negative.
" 9	8	98.7	..	26,900	.....	65.75	32.75	.25	.25	1	Throat better; typical desquamation beginning.
" 11	10	98.5	..	23,700	.....	56.50	39	3.50	0	1	Throat clear; heart action irregular; but no murmurs.
" 14	13	98.5	..	14,500	.....	.....	.....	....	....	....	Doing well; glands no longer enlarged.
" 17	16	98.6	80	22,100	5,168,000	64	34	1.75	.25	0	
" 21	20	99.5	..	13,100	.....	50.25	47.25	2	0	.50	Profuse desquamation.
" 25	24	98.4	85	12,300	.....	.....	.....	....	....	....	Cardiac condition improving.
" 29	28	98.4	..	12,600	.....	50	47.25	2	0	.75	
July 5	34	....	..	14,300	.....	50	45	4	0	1	Still some desquamation. Discharged well Aug. 7.

CASE 23.

SCARLATINA.

Girl, 5 years. Entered hospital June 11. No history obtained.

Physical examination: Robust; slight prostration; throat red, no exudate; papillae enlarged; lymph nodes in neck moderately enlarged; very brilliant punctate erythema on roof of mouth and over body. Soft systolic murmur over precardia. Spleen just palpable. Temperature 103.8° Urine: No albumin, June 11.

Date	Day Disease	Temperature	Hemaglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mono-nuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 11	3	103.8	..	13,800	.....	55	41.25	2.25	.75	.75	
" 13	5	104.1	80	16,400	5,152,000	87.25	11.75	1	0	0	Brilliant rash; throat very red but without exudate; enlarged cervical lymph nodes.
" 15	7	100	..	29,300	.....	64.50	30.50	4	1	0	Rash fading.
" 17	9	100	..	34,000	.....	56.75	40.75	2.50	0	0	Desquamation began yesterday.
" 20	12	99.7	70	16,200	.....	59	34.25	5	1	.75	Extensive desquamation; condition good.
" 23	15	98.5	70	18,600	5,280,000	54.50	42.50	2	0	1	Patient up.
" 27	19	98.5	..	13,000	.....	47.25	47	3.50	1	1.25	
" 30	22	98.9	..	11,600	.....	49.75	43	4	1	2.25	
July 5	27	....	60	13,700	.....	28	64	7.25	.25	.50	Still considerable desquamation.
" 8	30	....	60	24,100	5,260,000	54.50	40.50	4	0	1	No explanation of high white count.
" 12	34	....	..	12,600	.....	52	46	1.25	0	.75	
" 17	39	....	70	18,000	.....	53	46.25	.25	.50	0	Systolic murmur heard at base.
" 21	43	....	..	12,600	.....	41.50	56	2	0	.5	
" 25	47	....	65	16,600	.....	55.50	41	3.50	0	0	
" 30	52	....	..	11,900	.....	47	50.25	2.50	0	.25	Discharged well July 31.

CASE 24.

SCARLATINA.

Girl, 6 years. Past history: Pertussis, measles, and varicella. Present illness began June 9, with fever, vomiting, and rash.

Physical examination June 11: Well developed and nourished; no prostration. Tongue thickly coated, with enlarged papillae; throat red, with small amount of exudate on tonsils; lymph nodes in neck not enlarged; moderate punctate eruption on roof of mouth; bright on body (typical of scarlatina), already fading except on legs and back. Systolic murmur at apex. Temperature 101°. Urine: No albumin June 12 and July 8.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 11	3	101.2	75	17,500	5,572,000	77.75	16.25	5.75	0.25	0	
" 13	5	98.8	..	12,600	.....	60	32.75	6.25	0	1	Rash still bright on legs; no exudate in throat.
" 15	7	98.1	..	16,200	.....	45	47.25	4	0.25	2.50	Rash gone; moderate enlargement of lymph glands in neck; desquamation beginning on chest.
" 17	9	99	75	17,200	.....	65.25	34.75	0	0	0	Desquamation profuse.
" 20	12	98	..	12,600	.....	56.75	40	2	0	1.25	Patient up.
" 23	15	98.1	70	14,700	5,428,000	68.25	29.50	1.25	0	1	
" 27	19	....	..	16,700	.....	46	49.25	4.75	0	0	Still desquamating.
July 1	23	....	70	15,400	.....	48	43	8.75	0	0.25	
" 5	27	....	..	21,000	.....	63.75	32.75	3.50	0	0	No complications.
" 8	30	....	65	18,200	.....	55.25	38.25	5.50	0	1	
" 12	34	....	..	13,700	.....	63.50	31	5	0	0.50	
" 16	38	....	85	18,000	5,651,000	56.50	35	7.50	0	1	Heart action irregular.
" 19	41	....	..	15,200	.....	62	34	3.75	0	0.25	
" 23	45	....	..	9,800	.....	42.50	56.25	1.25	0	0	
" 28	50	....	..	11,200	.....	43	53	3	0	2	Discharged well July 31.

CASE 25.

SCARLATINA, ENDOCARDITIS.

Boy, 7 years. One brother ill at hospital with scarlatina (Case 27). Measles four years ago. Present illness began June 7, with vomiting, headache, and fever.

Physical examination June 10: Well developed and nourished; great prostration; delirious at times. Serous nasal discharge; throat red, with exudate on tonsils; papillae enlarged and red; large tender lymph nodes in neck; typical punctate erythema over body and on hard palate; most intense at flexures of joints and on legs. Heart: rapid, weak, and slightly irregular; enlarged to the left; systolic murmur at apex; pulmonary second sound accentuated. A few râles. Temperature 103.4°. Urine: June 10, slightest possible trace of albumin; June 14, 23, and July 14, no albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 10	4	103.4	..	20,600	.....	94	5	0	1	0	
" 12	6	100.2	80	21,500	4,508,000	88	10.50	1.50	0	0	Delirious.
" 14	8	100	..	18,800	.....	.....	.....	....	....	....	Better; desquamation begins.
" 17	11	99	..	23,300	.....	67.75	33.25	1.50	1	1.50	Marked improvement; pronounced desquamation.
" 19	13	98.7	..	13,600	.....	60.25	37.75	1	0	0	
" 22	16	98.5	75	13,400	.....	54.25	41.25	1.75	1.75	1	Cardiac dilatation; action irregular; systolic murmur.
" 26	20	98.6	..	13,300	.....	53.50	42	4.50	0	0	Continued improvement; still desquamating.
" 30	24	98.1	70	10,800	4,380,000	50	44.25	4.75	0	1	
July 5	29	99	..	8,100	.....	34	57.50	8.50	0	0	Serous inflammation of middle ear; paracentisis.
" 9	33	99.2	70	8,900	4,564,000	52.55	46.25	0.50	1	0	Slight discharge from ear; Aug. 18, heart still enlarged; somewhat irregular; loud blowing systolic murmur at apex transmitted to axilla. Discharged relieved.

CASE 26.

SCARLATINA

Boy, 6 years. One brother has scarlatina. Present illness began June 11, with vomiting.

Physical examination June 14: Mulatto boy, presenting only slight prostration; typical tongue, red with enlarged papillae; tonsils red and swollen, with a little exudate on back. Lymph nodes in neck enlarged, not tender, punctate eruption over body. Systolic murmur at apex. Urine: June 15, very slight trace of albumin. Temperature 100.2°.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 14	4	100.2	60	20,500	4,430,000	.....	.....	....	....	....	Rash still visible.
" 16	6	98.6	..	16,600	.....	47	50.25	1.75	0.50	0.50	Desquamation begins; rash gone; tonsils enlarged and reddened.
" 18	8	99	..	19,600	.....	59	39.75	1	0.25	0	Extensive desquamation.
" 21	11	99	65	25,000	.....	67	31.75	1.22	0	0	
" 24	14	98.5	65	17,400	4,416,000	58.50	37.50	2.75	1	0.25	Still much desquamation.
" 27	17	98	..	21,000	.....	53.25	45	0.75	0.75	0.25	
" 30	20	99	..	18,900	.....	44.50	50.50	4.75	0	0.25	
July 5	25	....	70	16,800	4,627,000	66.50	31.75	1.75	0	0	Still desquamating; patient improving rapidly.
" 9	29	....	..	16,400	.....	50.75	45	2.75	1	0.50	
" 12	32	....	..	16,100	.....	57	40	2.50	0	0.50	
" 16	36	....	70	19,400	4,688,000	38	60	0.75	0.75	0.50	
" 21	41	....	..	14,500	.....	40.25	58.25	1.50	0	0	
" 26	46	....	75	18,200	4,712,000	48	50.25	1.50	0	0	
" 30	50	....	..	13,700	.....	.....	.....	....	....	....	Discharged well, Aug. 3.

CASE 27.

SCARLATINA, ENDOCARDITIS.

Boy, 6 years (brother to Case 25). Measles four years ago. Present illness began June 9 with vomiting.

Physical examination June 10: Strong boy. Throat red; tonsils enlarged; some exudate; papillae large and red; punctate erythema on roof of mouth and skin, bright on extremities, fading on chest; lymph nodes in neck not enlarged. Heart not hypertrophied; systolic murmur. Urine: No albumin June 11, July 18 and 26.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 10	2	104.6	90	26,200	5,456,000	87.75	11.25	1	0	0	
" 12	4	100.8	..	24,000	.....	78.25	14.	7.75	0	0	Rash fading; conditions improving.
" 14	6	100	..	23,300	.....	68.75	23	8	0.25	0	Rash nearly gone; cervical glands much enlarged.
" 17	9	99.5	..	25,200	.....	79	16.50	4	0.50	0	Desquamation began on the 15th.
" 20	12	98.7	70	17,200	.....	73	24.75	2	0	0.25	Condition good.
" 23	15	98.5	80	20,000	4,949,000	71.50	25.50	2.25	0	0.75	
" 26	18	99	..	15,100	.....	57	39.50	2.75	0	0.75	
" 29	21	98.4	..	13,200	.....	46.75	44.25	7.50	0.25	1.25	Desquamation persists.
July 3	25	99	70	17,500	4,716,000	56.50	40.50	3	0	0	Heart examination, idem.
" 7	29	99	..	12,700	.....	44.75	51	3.75	0	0.50	
" 11	33	102	80	16,700	5,651,000	76.25	21.25	1	0.75	0.75	Follicular tonsilitis.
" 15	37	100.2	..	10,600	.....	.....	.....	....	....	....	Throat clear; July 21, typical signs of mitral endocarditis. Discharged relieved Aug. 16.

CASE 28.

SCARLATINA.

Boy, 3 years. Previously an attack of pertussis. Present illness: Onset June 10, with sore throat, vomiting, fever, and rash.

Physical examination June 11: Robust, no prostration. Little nasal discharge; papillae large and red; throat reddened, no exudate; no rash on roof; punctate eruption over body. Temperature 98.5°. Urine: no albumin June 12, 21, 25.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 11	2	98.5	85	12,800	5,068,000	44	50	4.75	0.50	0.75	
" 13	4	98.5	..	16,400	.....	79	19	2	0	0	Rash gone; desquamation on face.
" 15	6	98.5	..	18,300	.....	66	32	2	0	0	No symptoms; very mild case.
" 17	8	98.3	..	14,500	.....	68.50	29.50	1	0.25	0.75	Slight desquamation.
" 20	11	101	70	15,100	.....	82.25	15.75	1	1	0	Cervical lymph nodes enlarged and tender.
" 23	14	99.1	70	14,600	4,771,000	63.50	31.25	3.50	1	0.75	
" 27	18	98.5	..	18,600	.....	61.75	35.25	1.75	1	1.25	Still desquamating.
July 1	22	98.4	70	13,100	.....	35	63	1	0.50	0.50	
" 6	27	....	70	13,200	4,579,000	66.25	31.75	1	1	0	
" 18	39	....	65	10,400	4,864,000	46.25	51	2.75	0	0	Discharged well July 31.

CASE 29.

SCARLATINA; NEPHRITIS.

Girl, 11 years. Measles eight years ago. Present illness began June 10 with vomiting and sore throat.

Physical examination June 12: Vigorous child, considerable prostration. Throat red, no exudate; raspberry tongue; punctate rash on roof of mouth; brilliant punctate eruption of scarlatina over body, emphasized in the flexures. Heart rapid, not enlarged, systolic at apex. Pulmonary second sound accentuated. A few small lymph nodes in neck.

Date	Day disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast-cells	Remarks
June 13	4	103	..	18,400	.....	90	8.25	1.75	0	0	No albumin in urine.
" 16	7	100.1	75	15,800	4,339,000	.. ..	.. ..	..	..	..	Rash nearly gone.
" 18	9	100	..	19,200	.....	69.50	23.50	4	1	2	Desquamation on hands and feet.
" 21	12	99	..	17,000	.....	60.25	36	3	0	.75	Extensive desquamation.
" 24	15	102.2	..	15,000	.....	71.25	22.25	3.75	.25	2.50	
" 25	16	103.5	..	19,400	.....	85.75	13.50	.50	0	.25	Inflammation of glands in right side of neck.
" 28	19	98.4	70	22,600	4,492,000	81.25	16.25	1.50	.25	.75	Slight trace albumin in urine since 26th; glands less inflamed.
July 2	23	99.2	..	21,500	.....	83.75	13	2.75	.25	.25	Profuse desquamation; urine 12 oz.; trace albumin; symptoms of nephritis.
" 6	27	100	60	16,500	.....	76	16.50	5.50	0	2	Severe nephritic symptoms; urine typical of nephritis; rectal feeding.
" 10	31	101.1	..	29,600	.....	88	11.25	0	0	.75	Very sick.
" 14	35	100	70	19,700	4,792,000	88.25	11	.50	.25	0	Somewhat improved.
" 18	39	....	..	14,300	.....	71.50	28	.50	0	0	
" 22	43	....	55	13,500	.....	68	31.75	.25	0	0	Discharge from right ear since the 19th; improving.
" 28	49	....	55	12,300	3,277,000	61	37.50	.25	.25	1	Much improved.
Aug. 8	60	....	60	.. ...	3,824,000	.. ..	.. ..	.. ..	.. ..	.. ..	Discharged well Sept. 3.

CASE 30.

SCARLATINA.

Woman, 23 years. Pertussis in childhood. Present illness: Onset June 2, with sore throat, chill, vomiting, and fever.

Physical examination June 5: Well developed and nourished. Throat red; exudate on tonsils; papillae enlarged; rash on roof; fairly bright punctate erythema over body; lymph nodes in neck not enlarged. Heart normal. Temperature 103.3°. Urine: June 6, slight trace albumin with a few casts; July 8, slightest possible trace albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 6	5	101.5	..	9,500	.....	81	18	1	0	0	Vaccination.
" 8	7	100	..	9,300	.....	56	40.50	3.50	0	0	Rash normal.
" 11	10	98.4	..	6,900	.....	66.75	33.25	0	0	0	
" 14	13	98.5	85	6,400	4,624,000	54.50	42.50	2	0	1	Free desquamation beginning yesterday.
" 18	17	98.2	..	9,100	.....	45	43.25	10.50	0	1.25	
" 24	23	....	85	9,500	.....	70.50	25.50	1	2	1	Still marked desquamation. Discharged.

CASE 31.

SCARLATINA WITH RELAPSE.

Boy, 4 years. Pertussis eight months ago. Present illness: April 26, sore throat, high fever, and vomiting.

Physical examination, April 27: Papillae of tongue enlarged; thin membrane on both tonsils; profuse serous nasal discharge, cultures negative. No rash. Temperature 100.3°. April 30, a punctate eruption on body and hard palate, characteristic of scarlatina, appeared. The desquamation, which began May 2, was also typical. Disease ran normal course and on June 15 desquamation was over. June 19, fever of 102° and typical punctate erythema on whole body and hard palate, brilliant on thighs, feet, and lower abdomen. Fauces red, no exudate, papillae prominent; lymph nodes under left jaw enlarged. Urine: June 22, no albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 19	1	102	60	10,700	4,640,000	75	23.25	1.75	0	0	
" 21	3	103.3	..	12,000	.....	76	22.50	1.0	0	0.50	Rash bright on legs; beginning desquamation on face; throat reddened.
" 23	5	102	..	17,100	.....	69.25	29.25	1	0	0.25	Rash still visible on legs.
" 25	7	101	65	14,200	.....	50	43	6.25	0	0.75	
" 27	9	101.7	..	10,300	.....	59.50	36	4.50	0	0	Cervical lymph nodes enlarged; desquamation only on face.
" 29	11	100	65	18,400	4,272,000	62.50	30.50	5	0.75	1.25	
July 2	14	99.4	..	15,400	.....	56	37.75	5.25	0.50	0.50	Slight desquamation on legs and feet.
" 6	18	98.5	65	10,000	.....	45.25	51	3	0.25	0.50	Typical desquamation on feet and legs.
" 10	22	99.1	60	12,200	4,525,000	39	55.50	4.75	0.25	0.50	
" 17	29	98.5	65	6,900	.....	18.50	71	8.50	0.75	0.25	Discharged well Aug. 5.

CASE 32.

SCARLATINA.

Girl, 4 years. Measles one year ago. Present illness: Onset June 5 with headache, vomiting and fever.

Physical examination June 9: Robust child; extreme prostration, cyanosis and dyspnea; profuse purulent nasal discharge; tongue and throat typical of scarlatina; typical dark red punctate eruption on body; lymph nodes in neck greatly swollen with diffuse induration. Heart weak and rapid; moist râles. Spleen palpable. Temperature 100°. Cultures negative.

Blood examination June 9. White cells 18,500.

Temperature rose to 105° on June 10 and death occurred at 2 P. M. No autopsy.

CASE 33.

SCARLATINA; NEPHRITIS.

Girl, 5 years. Pertussis and measles one year ago. Present illness: Diphtheria March 23, treated in the hospital. April 21: Typical rash of scarlatina with strawberry tongue. Urine: April 21 and 28, no albumin.

May 9, edema of face; vomiting; urine contains over ½% of albumin. May 10, ½%, May 13, large trace; May 16, a trace with some blood and a few casts; less edema and vomiting. May 20; urine, slight trace albumin. Patient fed by mouth; no vomiting. Temperature 98.5°.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
May 20	29	98.5	..	19,800	.....	...	....	...	...	...	
" 28	37	99	..	20,000	.....	45.50	50	4.50	0	0	No albumin in the urine.
June 8	48	99	..	17,200	.....	49	42.75	8.25	0	0	Discharged well, June 12.

CASE 34.

TYPHOID FEVER IN CONVALESCENCE FROM SCARLATINA.

Girl, 8½ years. Measles, varicella, and pertussis six years ago. Present illness: Began May 20 with fever, cough, and rash on body.

Physical examination, June 10: Emaciated, pale, weak, apathetic; papillae large and red; throat somewhat red; on hand, forearms and abdomen desquamation of scarlatina; profuse and typical. Soft systolic murmur. Spleen not felt. Râles both cases. Urine, slight trace albumin.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Erythrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 10	22	102	..	5,400	.....	....	....	...	...	...	
" 13	25	103.6	..	6,000	.....	56	44	0	0	0	Symptoms suggesting typhoid.
" 18	30	98.5	60	5,600	.....	56	43.25	0.75	0	0	Rose spots; enlarged spleen; aggl. test positive.
" 26	38	101.3	60	18,900	.....	70.75	29	0	0	0.25	Adenitis colli.
July 1	43	102	..	8,100	.....	71	26	3	0	0	Relapse of typhoid.
" 9	51	100	55	10,400	.....	69.50	30	0.50	0	0	Rash nearly gone.
" 17	59	98.5	55	9,200	.....	65	33.75	1	0	0.25	Adenitis colli since the 11th; abscess opened and pus evacuated.

CASE 35.

SCARLATINA DURING CONVALESCENCE FROM MEASLES.

Boy, 5 years. Entered June 10, with measles, which ran the usual course. June 28, convalescence being established, the patient complained of sore throat, headache, and nausea.

Physical examination: Well developed and nourished boy. Erythematous rash over neck and chest, in places punctate. Throat reddened but without exudate; papillae of tongue swollen.

Date	Day Disease	Temperature	Hemoglobin	Leucocytes per c.mm.	Enthrocytes per c.mm.	Polynuclears	Mononuclears	Eosinophiles	Myelocytes	Mast Cells	Remarks
June 10	..	....	..	7,800	.....	....	....	...	...	...	
" 14	..	....	..	6,700	.....	....	....	...	...	...	
" 17	..	....	..	9,800	.....	....	....	...	...	...	
" 20	..	....	..	11,400	.....	....	....	...	...	...	
" 23	..	99.3	..	17,000	.....	73.50	23.75	2.5	0	0.25	
" 26	..	99.5	75	6,700	.....	64.25	30.75	4.25	0	0.75	
" 28	1	104	..	21,600	.....	79.25	19	1.75	0	0	Typical signs and symptoms scarlet fever.
" 30	3	99.2	65	25,600	4,644,000	89.75	8.75	1.25	0	0.25	Brilliant rash.
July 2	5	101	..	18,600	.....	66.25	27	6.75	0	0	Desquamation begins, but rash still visible; conjunctivitis.
" 3	6	99.3	..	18,800	.....	67.25	29	3	0.25	0.50	
" 5	8	99.8	..	19,300	.....	59.75	35.25	4.50	0	0.50	
" 8	11	100	..	13,800	.....	56.75	38.25	5	0	0	Euphoria.
" 11	14	98.5	70	10,400	5,238,000	51.25	43	5	0	0.75	Typical desquamation; good convalescence.
" 15	18	99.4	..	13,000	.....	47.75	47.75	3.50	0	1	
" 19	22	99	65	9,500	.....	40.75	53	5.50	0	0.75	
" 25	28	98.5	75	13,600	5,108,000	47.50	46	6.50	0	0	Discharged well Aug. 20.

SUMMARY OF RESULTS.

For purposes of blood examination several authors (Kotschetkow, Bowie, and Klotz), have divided their cases into three groups, i. e., 1) mild, 2) moderate, and 3) severe, usually fatal, and grouping ours thus we find 16 of the first, 11 of the second, and 5 of the third type. A close examination of our cases thus grouped, however, fails to give the constant results obtained by these authors, and it seems to us more reasonable to establish standards for the average case of scarlatina, noting the exceptions when the disease is of the very mild or extremely severe type.

As Tileston has emphasized, any attempt at definite deduction regarding the number of white cells occurring in the course of any disease must take into consideration the normal variations from year to year in infancy and early childhood. He states that the normal number of leucocytes in infancy varies from 10,000 to 15,000 per c.mm., dropping to about 12,000 per c.mm. in the second year and slowly decreasing to reach the normal

adult value (7,500 to 10,000 per c.mm.) at the tenth year. In children the lymphocytes are somewhat increased with a corresponding decrease in the polynuclears as compared with adults, while slight or unknown causes produce marked changes. Bearing these facts in mind we sought to tabulate our results by years, but found only insignificant differences, and therefore combined all cases over two years of age. In general the youngest children showed slightly higher counts, with somewhat more abrupt changes in the curve and in a few instances a high percentage of mononuclear cells.

HEMOGLOBIN.

The changes in the percentage of hemoglobin are somewhat irregular, but commonly the value is at the beginning normal, later showing a tendency to a gradual decrease. In several of the mild as well as the moderately severe cases no appreciable diminution could be made out while in others a loss of from 5 to 20 per cent was noted; the severe cases, so far as studied, were uniformly accompanied by a loss of from 10 to 25 per cent.

Complications, especially diphtheria and nephritis, often produce an abrupt loss in a few days of from 5 to 20 per cent. Our experience in this regard agrees with that of Widowitz as opposed to Reckzeh who asserts that complications exert no influence in lowering the hemoglobin.

In uncomplicated cases the loss is very gradual occasionally reaching its lowest point only after several weeks and more slowly mounting up to normal during convalescence.

ERYTHROCYTES.

With our series of 35 cases the changes in the red cells were much less definite and constant than those described by several authors. Every case of Reckzeh showed variations in the size of the erythrocytes and many a slight polychromatophilia; Berg mentions a count of 3,500,000 c.mm. as frequent; Kotschetkow says in all his cases the red count sank to 3,000,000 per c.mm. or lower, increasing again not earlier than the sixth week; Hayem's loss in the number of red cells was about 1,000,000, the lowest count coinciding with the fall of temperature; Mackie found three and one-half to four million cells in about one-half of his 25 cases.

Unfortunately, as will be seen upon examination of the foregoing tables, the red counts were made with much less regularity than the white counts and consequently less definite deductions are possible. At the onset the number of erythrocytes was almost invariably normal (4,300,000 to 5,600,000 per c.mm.). During the first few weeks of the disease their course varies with the severity of the condition, in very mild cases usually not diminishing at all or even increasing, in the cases of moderate severity sometimes falling from 100,000 to 700,000 per c.mm., and in the severe types sinking much more abruptly and constantly though in no instance more than 700,000. Among the uncomplicated cases the lowest count recorded is 3,700,000 per c.mm., Case 8, a somewhat debilitated, rhachitic child of two and one-half years. Rarely in the cases with grave symptoms, either from a prolonged course or complications, very moderate variations in the size and shape of the cells were evident and in one or two such, a few normoblasts were found, but no other abnormalities in the erythrocytes showed themselves.

LEUCOCYTES.

Total count.—The chief interest and importance in the blood changes in scarlatina center about the white cells and here our records, comprising 366 counts with 348 differential determinations are, we believe, more complete than any recorded previously. Only one observation was possible previous to the onset of symptoms hence we are able to offer no data as to the behavior of the leucocytes during the incubation stage.

Our tabulated results at first sight give the impression of a very disappointing lack of uniformity in the course of the leucocytes, but after eliminating such counts as may be influenced by complications, and a few striking exceptions in the very mild and the fatal cases, the counts are surprisingly uniform. The series shows a constant leucocytosis, as a rule of marked type in the cases of moderate severity, somewhat less so in both the mild and the very severe fatal cases. The degree of leucocytosis varies so much with the stage and severity of the disease that any general exact statement of the white count in scarlatina is impossible; therefore, in an attempt to represent the course of the white

count in the various stages of the disease we have combined the above tabulated results into a composite curve (Table 1), excluding all such counts as appear to be influenced by complications or other conditions. Though obviously in some respects inaccurate, and differing somewhat from the course in individual instances, it clearly represents in the most accurate manner possible the average. It will be seen on reference to Table 1 that the leucocytosis rises somewhat during the first two days (16,000 to 17,000 per c.mm.), then more suddenly on the third to its maximum (23,000 per c.mm.), when it slightly less abruptly falls on the fourth and fifth, to rise again to 21,400 on the 11th. Subsequently the general course is irregularly downward, till at the end of the third week the count becomes about 12 to 14,000 per c.mm. During the fourth and fifth weeks the curve is irregular, but with a general tendency upward. The white count finally approximates normal limits in the sixth or seventh week. To this general course are several striking exceptions in the cases tabulated in the earlier part of this paper. The cases of a very mild type as a rule show a less intense leucocytosis and of shorter duration than the above, while many of the very severe ones, on the other hand, give much higher values, and are sustained for a longer time. Case 30 of our series, an adult with very mild symptoms, throughout the course of the disease maintained a normal leucocytosis. In Case 2 the white cells rose to 26,000 per c.mm. on the third day, falling to 13,900 on the sixth; in Case 3 to 44,000, also on the third day, sinking to 26,000 on the sixth; in Case 27 to 26,000 on the second day and subsequently falling very gradually to 15,000 on the 18th day. These three cases were all young children in whom the rash was brilliant, but the symptoms very mild. Cases 17, 18, and 19 strangely enough all gave their maximum leucocytosis on the 11th day, the counts being respectively 47,000, 31,900, and 25,700 per c.mm. since all are of the moderately severe type, no explanation is evident from the clinical side, in two cases the temperature being normal and the course in each case favorable. The maximum count in Case 8, occurred on the eighth day, rising from 16,600 on the fifth to 34,600 at the end of three days. The

following increase in the white count in Case 3, during the later course of the disease, and without apparent cause, is interesting:

Day of Disease	White Count
24	23,900
30	34,300
36	79,500
43	46,900
49	21,600

The above count of 79,500 c.mm. is the highest noted in our series in the uncomplicated cases. Reckzeh mentions one of 41,000, Klotz one of 78,000, while Klein made a single count of 80,000.

Kotschetkow, Reckzeh, and Sacquépée agree in their opinion that the amount of leucocytosis bears no direct relation to the severity of the disease; while Mackie, on the other hand, records exactly opposite findings. Though not absolutely constant, our cases, within certain limits, give a definite relation as mentioned by Mackie. The time of duration of leucocytosis is generally conceded to bear a direct relation to the gravity of the symptoms. The maximum leucocytosis seems to correspond quite closely in time with the highest temperature, but appears from one to two days earlier and persists from a few days to several weeks longer, and during the course of the disease is not influenced by the character of the fever curve. All writers appear to be in agreement on this point.

Like Reckzeh, we have never seen the secondary rise in the number of leucocytes which Türk described as occurring at the height of the fever.

Differential count.—The course of the different kinds of leucocytes presents considerable variation in a few individual cases, as is true of the total leucocytosis, yet, considered together, the cases conform to a uniform type. The polynuclears are, during the first few days of the disease, relatively much increased, reaching the maximum (80 to 95 per cent) on the second to eighth day, to fall abruptly, then gradually to normal or subnormal at the end of the third to sixth week. Absolutely these cells at first take almost precisely the same course, but later fall more rapidly. During the first week the absolute value runs parallel with the

total leucocytosis, but subsequently sinks more abruptly, due to the increase in the mononuclears. Never, however, in contrast to the percentage value, does the number of neutrophiles become subnormal. Both relatively and absolutely the mononuclear cells take a direction complementary to the polynuclears. From a normal percentage at the onset they quickly fall on the second to fourth day to even 4 or 5 per cent in extreme cases, rise to normal during the next few days, and for the following few weeks more slowly to 50 per cent or higher (Case 4, 74.75 per cent). Convalescence is thus characterized by a mononucleosis which persists for many weeks. As a rule the absolute number of mononuclear leucocytes is at first slightly increased, and throughout the remainder of the disease greatly so. With the constantly falling leucocytosis after the initial rise it is obvious that the actual increase in the number of mononuclears must be less marked than the rise in percentage. It will be readily seen from the above that the initial leucocytosis is chiefly a polynucleosis, while later the mononuclears participate more and more in the increase of white cells.

The eosinophiles in our experience, are in the beginning either absent or much diminished but soon rise to slightly above normal values, often on the fourth or fifth day as high as 5 or 6 per cent. Even during convalescence a continued or temporary high value is sometimes met. In Case 1 the eosins were relatively increased throughout, on the ninth and eleventh day reaching 11 per cent; Case 14 showed 11 per cent on the fifth day; Case 30, 10 per cent on the seventeenth day. These changes just described agree in their essential features with the results published by previous workers.

In Table 1 we have included, beside the leucocyte curve, the composite curves of the absolute values of the three main types of white cells, and in Table 2 their relative values. Table 3 represents the results of the differential counts in Case 35.

No especial changes in the percentage of mast cells has been noted in this series except occasionally a slight decrease in the early stages of the disease followed in convalescence by a material increase.

TABLE 1.

COMPOSITE CURVES SHOWING THE COURSE OF THE TOTAL LEUCOCYTOSIS AS WELL AS THAT OF THE VARIOUS TYPES OF WHITE CELLS IN SCARLATINA.

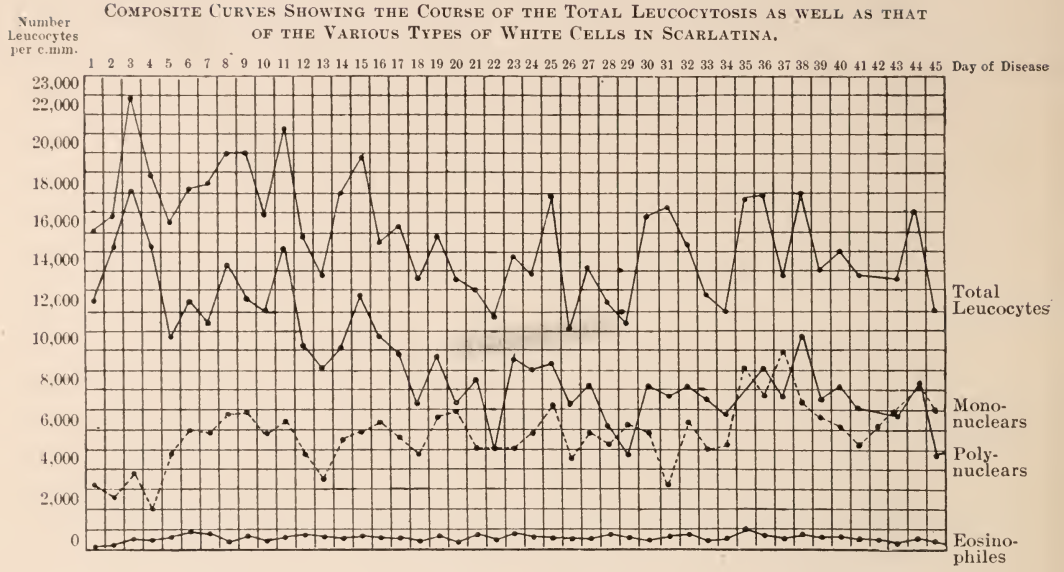


TABLE 2.

COMPOSITE CURVES SHOWING THE COURSE TAKEN BY THE RELATIVE VALUES OF THE POLYNUCLEARS, MONONUCLEARS AND EOSINOPHILES IN SCARLATINA.

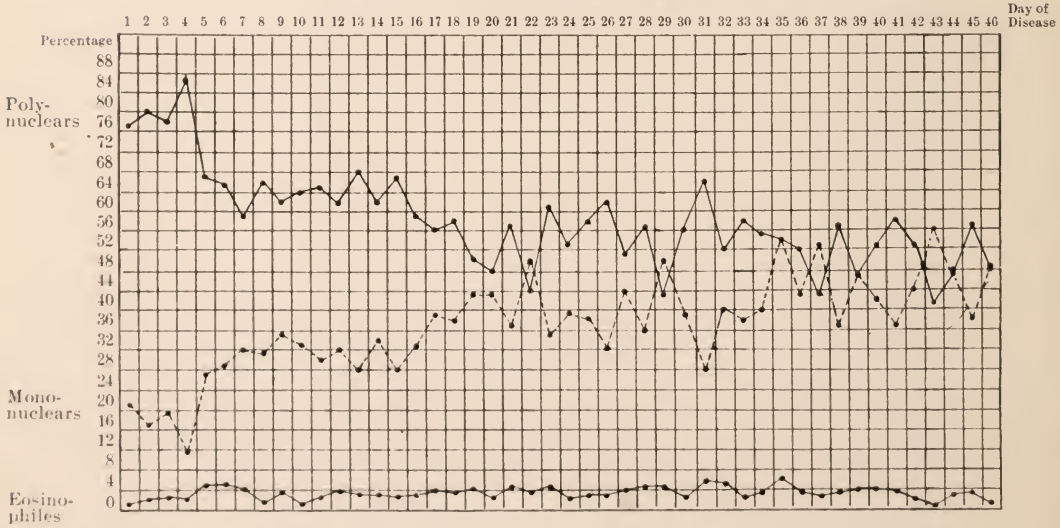
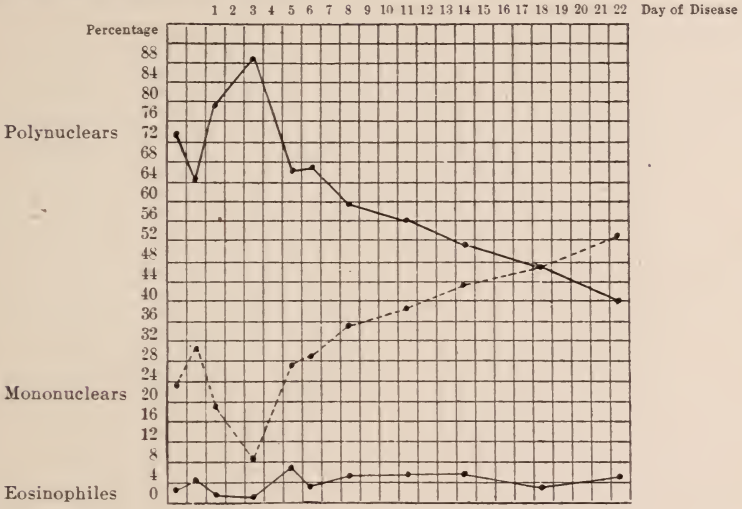


TABLE 3.

CASE 25.—This chart represents the variations in the polynuclears, mononuclears, and eosinophiles throughout the disease.



At the time of making the differential counts, the blood platelets in all cases were noted as diminished, normal or increased. Their number seems to be extremely variable and without constant relation to the period or severity of the disease, although in several instances they were distinctly increased during early desquamation. Türk states positively that the plates are increased during this period.

COMPLICATIONS.

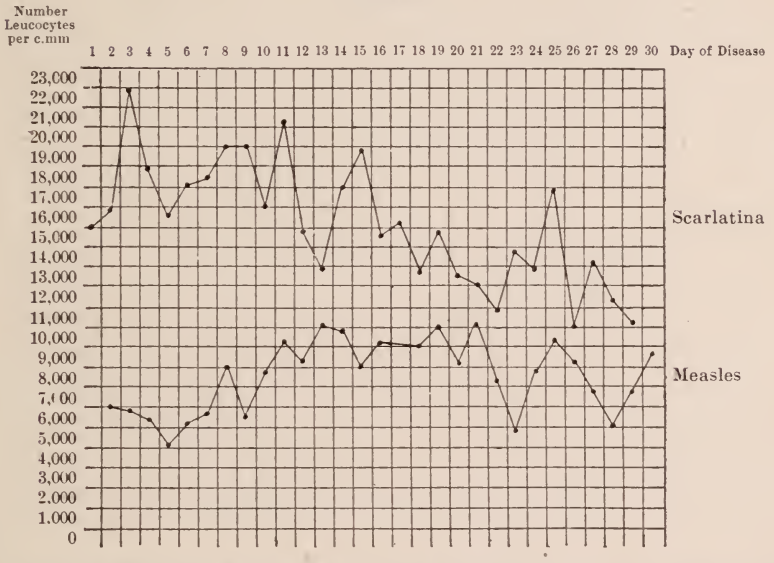
Our study of the diseases occurring in the course of scarlatina with reference to any possible influence on the blood comprises the following:

Diseases.	Number of Cases.
Measles	1
Abscess	5
Endocarditis	2
Otit's media purulenta	3
" " serosa	1
Diphtheria	4
Acute Nephritis	3
Cervical Adenitis	10
Typhoid	1

On the whole the results are surprisingly negative, the cases of measles, local abscess, endocarditis, otitis media serosa, and typhoid producing no definite alterations in the blood. Of the remaining, two cases of otitis media purulenta (8, 19), gave a slight increase in the leucocytosis with a corresponding rise in the percentage of polynuclear leucocytes. One case of diphtheria (10) evidently produced similar changes but of only a very moderate degree. Case 9, following immediately the onset of symptoms of nephritis which developed on the 22d day of the disease, showed an increase in the number of leucocytes from 17,500 to 20,800 with an increase in the percentage of polynuclears from 67.50 to 72.50. Three days later the white count rose to 25,900 and the neutrophiles to 74.25 per cent. With the subsidence in the symptoms both diminished rapidly. Very similar changes accompanied the course of the nephritis in Case 29 but since otitis media and cervical lymphadenitis were also present no deductions are possible. Of the 10 cases of cervical adenitis occurring in the course of scarlatina, three (12, 27, and 28) apparently produced a marked increase in the white count and neutrophiles. Various complications and sequelae, by prolonging the course and adding to its severity naturally, induce a somewhat greater degree of anemia than is commonly present. Concerning the influence of these intercurrent diseases on the blood in scarlatina the various authors are somewhat at variance. Widowitz mentions the possibility of a sudden and marked diminution in the percentage of hemoglobin following the onset of nephritis. Kotschetkow and v. Berg believe that complicating lymphadenitis, otitis media and nephritis exert no influence on the number of leucocytes. Sacquépée in one case each of nephritis and adenophlegmon found a hyperleucocytosis accompanied by a polynucleosis, while in a case of mumps a mononucleosis developed. Two cases of nephritis observed by Reckzeh produced no obvious effect on the blood while endocarditis, diseases of the bones, gumboils and adenitis did induce a hyperleucocytosis. Adenitis, otitis and nephritis in Bowie's experience all augment the leucocytosis. Klotz states that lymphadenitis, arthritis, varicella and otitis show an increase of

4,000 to 12,000 in the number of white cells, the polynuclears showing the greatest change, and nephritis either an increase or decrease.

TABLE 4.



Composite leucocyte curve based upon 350 counts in 34 cases of scarlatina and 200 counts in 28 cases of measles, showing the striking difference in the course of the leucocytosis in the two diseases.

THE BLOOD IN MEASLES AND SCARLATINA.

Tileston has already discussed the differences in the blood in scarlatina and measles. The contrast in the course of the leucocytosis is very strikingly brought out in Table 4, the composite curves being made up from the 35 cases of scarlet fever included in this investigation, and the 28 of measles published by Tileston. It will be noted that the curves are almost complementary throughout, in the first case a persistent though gradually falling hyperleucocytosis followed by a gradual rise to normal. It is in this regard that the blood examination may occasionally be of considerable value in differentiating between these two diseases. Otherwise we must admit that the study of the blood in scarlet fever is of no advantage in diagnosis.

CONCLUSIONS.

1. The blood of scarlatina in children differs from that in adults only in proportion to the differences in normal blood at the different ages.

2. A slight secondary anemia is the rule in all but the very mild cases, varying directly with the severity and duration of the disease. The fall in hemoglobin is from 5 to 25 per cent and in the erythrocytes from 100,000 to 700,000 per c.mm. Both return to normal after a period of several weeks.

3. A hyperleucocytosis almost invariably accompanies the disease and runs a characteristic course. Rising abruptly on the second to eighth day (18,000 to 40,000 per c.mm.) the count falls rapidly for a few days then more gradually to reach normal in convalescence, usually at the end of from three to six weeks.

4. During the period of invasion and eruption the polynuclear leucocytes are both relatively and absolutely increased but decrease gradually with the fall in the leucocytosis till convalescence when they may become relatively, though never absolutely, below normal. The mononuclears take an exactly opposite course. With the onset the eosinophiles disappear entirely or are greatly reduced, to rise above normal when defervescence begins. This eosinophilia persists until late convalescence. Myelocytes are often seen in small numbers as in all infectious diseases.

5. Complications, with a few exceptions, exert no influence upon the course of the blood. If severe they may increase the anemia and in a few instances (nephritis and diphtheria) even produce a rise in the leucocytosis.

REFERENCES.

VAN DEN BERG. *Archiv. f. Kinderheilk.*, 1898, 25, p. 321.

BOWIE, J. M. *Jour. Path. & Bact.*, 1902, 8, p. 82.

FELSENTHAL. *Archiv. f. Kinderheilk.*, 1892–3, 15, p. 82.

HALLA, A. *Ztschr. f. Heilk.*, 1883, 4, p. 198.

HAYEM, G. *Du sang et de ses altérations anatomiques.* Paris, 1889, p. 214.

KLEIN. *Volkmann's Samml. klin. Vorträge.* N. F., 1893, p. 825.

KLOTZ, O. *Jour. Infect. Dis.*, 1904, 1, p. 404.

KOTSCHETKOW, VON W. *Wratsch*, 1891, No. 41. (Ref. von Abelmann.) *Jahrb. f. Kinderheilk.*, 1891, 36, p. 409.

LEICHTENSTERN. *Untersuchungen über den Gehalt des Blutes in gesunden und Kranken Zuständen.* Leipzig, 1878, p. 77.

Mackie, F. P. *Lancet*, 1901, 2, p. 525.
Pée, H. C. *Untersuchungen über Leukocytose.* Inaug.-Dissert., Berlin, 1890.
Pick, G. *Prager med. Wchnschr.*, 1890, 15, p. 303.
Reckzeh. *Ztschr. f. klin. Med.*, 1902, 45, p. 201.
Rieder. *Beiträge zur Kentniss der Leukocytose.* Leipzig, 1892.
Rille, H. *Archiv f. Dermat. u. Syphil.*, 1892, p. 1028.
Sobotka, J. *Ztschr. f. Heilk.*, 1893, 14, p. 415.
Sacquépée. *Archiv. de méd. exper. et d'anat. path.*, 1902, 14, p. 101.
Tileston, W. *Jour. Infect. Dis.*, 1904, 1, p. 551.
Türk, W. *Klinische Untersuchungen über das Verhalten des Blutes bei acuten Infectionskrankheiten.* Wien, 1898.
Widowitz, J. *Jahrb. f. Kinderheilk*, N. F. 1887, 27, p. 384.
Zappert, J. *Ztschr. f. klin. Med.*, 1893, 23, p. 227.

NOTES ON A CASE OF HEMATOCHYLURIA, TOGETHER WITH SOME OBSERVATIONS ON THE MORPHOLOGY OF THE EMBRYO NEMATODE—FILARIA NOCTURNA.*†

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THE diagnosis of many cases of filariasis in which the adult parasites are inaccessible must depend upon the identification of the embryo nematodes. Most of the published pictures of *Filaria nocturna* have been drawn, apparently, from stained preparations and all the high power photomicrographs we have seen depict the parasite in a greatly degenerated condition and do not present the morphological details observed in a fresh preparation. The study of a case of hematochyluria during the past six months and the accessibility of a Zeiss photomicrographic apparatus have given us the opportunity of presenting the accompanying illustrations together with an abstract of our notes on the case.

ABSTRACT OF CLINICAL HISTORY.

On May 30, 1904, O'Saya, a Japanese girl, 22 years old, came under our observation. She came to Manila four years ago from a village near Nagasaki. The patient was emaciated, pale and weak and complained of passing bloody and milky urine and of attacks of abdominal pain referred chiefly to the right lumbar region. The abnormal urine was first noticed in August, 1903. This disappeared spontaneously in three months and she had no further trouble until the present attack. She claimed to have always drunk boiled water or tea, and she gave a history of previous good health. The patient was placed in the Manila Civil Hospital where during the evening and at night she was protected by a mosquito net.

She was passing a considerable amount of milky, peach-colored urine, sometimes quite bloody, which upon cooling contained large and small clots of reddish and yellowish jelly-like material. These fibrinous clots were sometimes passed through the urethra and occasioned some pain. In the centrifugate a number of filaria-like organisms were found but as none could be

* Received for publication February 10, 1905.
† Presented before the Manila Medical Society, September 12, 1904.

found in the peripheral circulation during the day or at night the patient was put on tonics and vesical irrigations of boracic acid until the end of June, when urotropin one gm. t.i.d. was given and the douches changed to bichloride of mercury 1–10,000 and morphine was given hypodermically for the pain.

At 9 P. M. July 23, filaria, resembling those occurring in the urine, were found in the blood from a finger. The patient was kept in a bed the foot of which was elevated and received just enough food, without fats, and liquid to sustain life, and a very weak solution of adrenalin chlorid was injected into the bladder and allowed to remain.

From August 1 to 15, the adrenalin was given by mouth, 10 to 15 drops of a 1–1000 solution every four hours during the daytime, 40 to 50 drops per day. On August 15 this treatment was stopped. At this time some swelling of the right thigh developed but subsided after a few days. The patient remained in the elevated bed until the middle of October.

On August 29, methylene blue, 0.12 gm. every four hours was given by mouth. This was stopped on September 4, on account of the occurrence of violent emesis.

At the suggestion of Dr. W. E. Musgrave we attempted to "sensitize" the adult parasites by the administration of quinine followed by the exposure of the body of the patient, through the lumbar region, to the X rays. She was given 80 to 90 grains of quinine sulphate during 48 hours followed by X ray exposures of five minutes, with the tube 18 inches away. Quinine having been administered daily, these exposures were performed at 2 to 3 P. M. on September 8, 10, 11, 12, 14, 16, 17, 18, and again after cinchonizing as before at 9 P. M. on September 29, and 30.*

On October 2, the skin over the chest and abdomen became reddened and hot. A chill and left pleurisy developed. Paracentesis produced about 600 c.c. straw colored fluid on October 8. Skin scarlet all over body. All this time the urine remained thick and bloody, but on October 10 became normal and has remained so. The temperature throughout, except during the attack of pleurisy, remained about normal, 97°–99.4° F. in the morning and 98°–99.4° F. in the evening.

Until the pleuritic attack the patient had gained 25 per cent in weight and general appearance, and although an evening temperature of 1°–3° F. persisted until October 22, she regained strength so rapidly that on October 29 and 30 the X ray was again applied for fifteen minutes, after quinine, with the bulb five inches away.

Although the patient has been at home and walking about for the past two months her chyluria has not returned. The living embryos still persist in her blood, and hence it is altogether likely that the treatment had no effect upon the adult parasites.

*Unfortunately we are unable to state the exact hardness of the X ray bulbs. In order to obtain a clear radiograph of the bones of the pelvis with this apparatus an exposure of 15 minutes with the bulb at a distance of five inches from the skin surface is necessary. Filarial embryos in a thin layer of blood, collected after cinchonizing, exposed to the rays for five minutes with the bulb 16 inches away are not killed but they squirm about in a very excited manner.

SPECIAL FEATURES OF THE CASE.

1. *The Urine.*—The urinalyses have yielded the usual findings in such cases, excepting our failure to extract fatty matters in appreciable quantities. The bloody, milky urine never altered its appearance on prolonged shaking with ether, even after it was made alkaline with sodium hydroxid, and the evaporation residue seemed to consist of other than fatty extractives, though in one instance a trace of fat was found by testing for glycerin. Its milky appearance may have been partly due to the considerable number of leucocytes it contained. The amount of albumin varied between 0.33 and 0.6 per cent. For example, an analysis by Mr. C. L. Bliss on August 26, gave: Quantity, 675 c.c.; sp. gr. 1026; reaction acid but turning, kept at 30°; albumin 0.33 per cent, average of five tests by Esbach's method; fat, trace, glycerin test.

2. *The Blood.*—Four days after admission a blood count gave 3,100,000 reds and 6,000 whites. The anemia almost disappeared as the general condition of the patient improved while at rest with the hips elevated. The excessive loss of blood did not continue for long and the anemia did not reach the grave character of such cases as Herrick[1] described as due to repeated losses of blood from hemorrhoids.

On July 30 the number of parasites per c.c. in the patient's peripheral circulation was calculated. In order to obtain drops of blood of known volume, the method of collecting it and estimating the number of parasites per c.c. used by Lathrop and Pratt was employed. Three equal-sized drops of blood were taken every two hours, beginning at 10 A. M. and ending at 6 A. M. on the following day. The average number of parasites present was then determined by counting the stained filaria on a mechanical stage. The chart shows the rise and fall in the number of filaria per c.c. present during the different times of the day and night.

In 1901, Calvert,[2] working in Manila on Filipino prisoners of war, found four cases of filariasis—lymphatic varices and hydro-

[1] *Jour. Amer. Med. Assn.*, Sept. 27, 1902; 39, p. 767.

[2] *Johns Hopkins Hosp. Bull.*, 1902, 13, p. 133.

cele. No description of the parasites is given, but they were of the nocturnal variety and probably the embryos of *Filaria bancrofti.* By means of extensive blood examinations of three cases he showed the presence of a decided eosinophilia, which was most marked at the time when the embryos were absent from the peripheral circulation. Trichinosis was excluded, but apparently no examination was made for uncinaria.

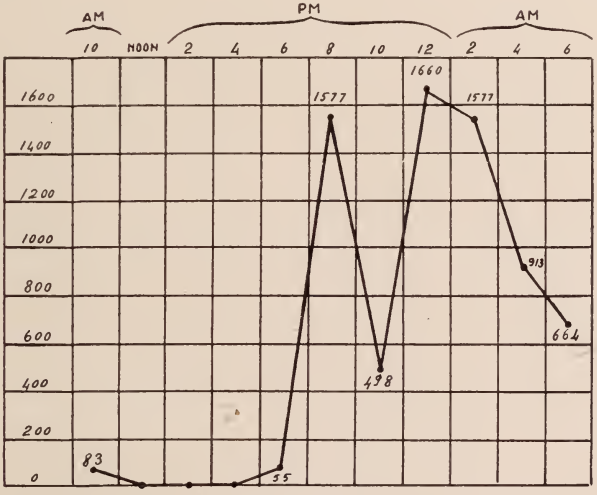


Fig. 1.—Chart showing the rise and fall in the number of filaria in the patient's peripheral circulation during different times of the day and night.

The figures in the left hand column arbitrarily represent the number of filaria per c.c. of blood, while those within the ruled squares indicate the calculated number of filaria per c.c. of blood.

In our case no such hourly counts were made. The eosinophiles varied from 6 per cent (10 A. M.) to 12 per cent (12 P. M.) and were most numerous in the peripheral circulation at the time when the embryos occurred in greatest numbers, as follows:

July 28, 10 A. M. Reds, 4,340,000; whites, 11,000.

P and T*	55%
E	6
B	4
LM	22
SM	13
	100

100 leucocytes counted; one normoblast and no filaria seen.

*P and T = Polynuclear and transitional leucocytes. E = Eosinophiles. B = Basophiles. LM = Large mononuclears. SM = Small mononuclears.

August 9, 8 P. M. Reds, 4,290,000; whites 6,000.

Differential.

8 P. M.		12 P. M,	
P and T	72.00%	P and T	71.50%
E	10.50	E	12.00
B	1.50	B	1.50
LM	2.50	LM	5.00
SM	12.50	SM	10.00
	100.00		100.00
200 leucocytes counted.		200 leucocytes counted.	

Number of Filaria per c.c.

8 P. M.	12 P. M.
489	1,079
Calculated from the average number in two slides.	Calculated from the average number in two slides.

On the morning of August 10, the patient was given an ounce of magnesium sulphate and her stool carefully examined for signs of intestinal parasites. Nothing but a few ova of *Trichocephalus dispar* was found. We believe that trichinosis can be excluded and are not aware that the eosinophilia can be influenced by the *Trichocephalus dispar* which the patient harbors.*

According to Manson[1] the presence of blood in the urine in such cases is not due to the rupture of blood vessels, but to "the formation of blood corpuscles in the lymph long retained in the varicose vessels." Our failure to alter the sanguineous character of the urine by the administration of adrenalin, locally and by the mouth, seems to support this idea. On the other hand, the development of anemia and the presence of a few normoblasts in

* REMLINGER (Constantinople) (*Compt. Rendu Soc. Biol.*, 1904, 57, p. 76) has recently noted an eosinophilia of 43 per cent in a case of multiple infection with the Medina worm. He says: "Elle est également à rapprocher de l'éosinophilia observée la *Filaria sanguinis hominis*, la *Filaria Loa*, la *Filaria Immitis*, du Chien, etc., etc."

MANSON (*Brit. Med. Jour.*, Sept. 1, 1900, 2, p. 536) has expressed the belief that a large number of filarial embryos in the peripheral circulation indicates a multiple infection with adult parasites. Apparently there is no definite information as to the fate of the embryos, but Bancroft computes their life duration at a few months. To our knowledge they have not been observed in the dead condition in the blood of man except when killed by some form of medication as in Scheube's case which was treated with picric nitrate of potash. It seems possible that they may accumulate in the blood and, if so, large numbers would merely indicate that the case was of long standing. The grade of eosinophilia together with an enumeration of the embryos by the method of Lathrop and Pratt, especially if supported by a postmortem or post operative search for the adult parasites, would throw light on this point.

[1] *Tropical Diseases*, 2d ed., 1903, p. 581.

the peripheral circulation would seem to indicate that at least a portion of the loss occurred through capillaries torn during the rupture of dilated lymph vessels, as is suggested by Scheube.[1]

3. *The embryo nematode* has been well described by Manson and our own study has been greatly influenced by his excellent descriptions. A brief description made on July 30 may be inserted here.

A fresh preparation was made at 10 P. M., ringed with vaseline and a filaria watched for some time. It underwent the usual movements of coiling, uncoiling and sliding forward and backward with its sheath. At about the junction of the middle and posterior thirds there could be seen an irregularly elongated viscus-like organ, which seemed to be composed of a granular tissue that was almost whitish by transmitted light. In about two hours the motions were reduced to very slight squirming movements. The outer contour of the filaria was clear cut, but within its lateral borders the serrated edges of the transverse fibres of the musculocutaneous layer could be traced. A little more than half-way between the anterior end and the viscus-like organ there was a refractile V-shaped papilla with its apex turned toward the lateral border of the filaria. The head end was observed with the $\frac{1}{12}$ oil im. and comp. oc. 8, and at its extreme tip a notched retractile lip could be seen. Owing to the rapidity of the retractile movements the number of notches was indistinguishable. In addition there was a short, refractile, needle-like process, which was seen to be projected and withdrawn. A few minutes later, when the movements had become slower, the lip, when retracted, showed at least three refractile teeth-like projections, and the needle-like spicule was seen to be projected at about the level of the middle tooth. (See Figs. B and C, Plate 14.)

By watching carefully the contractions and relaxations of the circular muscular fibers, three narrow, refractile, and sinuous duct-like threads could be traced backwards until they united with the anterior end of the viscus-like organ (see Fig. 2, Plate 13, and Fig. B, Plate 14). The exact manner in which they terminated

[1] *Die Krankheiten der warmen Länder*, 1904 (with complete bibliography).

anteriorly could not be made out. No particular structure could be distinguished posterior to the viscus-like organ, excepting a refractile V-shaped papilla, like the anterior one, and situated on the same side of the body at a point slightly posterior to a point half-way between the hind end of the viscus and the tip of the tail. The loose, transparent sheath could be seen projecting beyond the posterior end, but not beyond the anterior end of the parasite. In six hours ecdysis was not complete, the viscus-like organ had disappeared, and refractile granules began to make their appearance in the protoplasm. The parasite was measured just before granular degeneration set in, and was found to be: Length, 0.31 mm.; greatest breadth, 0.0075 mm.; from anterior tip to anterior end of viscus, 153 μ; length of viscus, 49.5 μ; from posterior end of viscus to tip of tail, 114.75 μ (Zeiss $\frac{1}{12}$ oc. microm. 3.) The average of four measurements is 0.327 mm. by 0.0074 mm.

We have no new morphological details to add, excepting the three duct-like threads which connect the viscus-like organ with the head end of the embryo. These must be looked for as soon as the motions of the parasite become slow enough to permit the use of an oil-immersion lens, for the granular degeneration, which sets in soon after motion ceases, obscures all finer details.

4. *Photographing the embryo.*—It is quite difficult to obtain good high-power photographs of the live filaria, and Mr. Martin's success followed only the most persistent efforts. The ray-filter must be dispensed with and the photograph taken very soon after the embryo is exposed to the rays of the electric arc, as it undergoes rapid granular degeneration and its motions cease much sooner than when subjected to ordinary daylight.

Prevalence of the disease in the Philippine Islands.

There seems to be little positive information on this point. Calvert's cases represented northern and southern Luzon. Scheube included this island, but none of the southern islands of the group, in his map showing the geographical distribution of filariasis. We have not had time to go into this side of the subject thoroughly, but inquiry shows that physicians who have been

in Manila for from 10 to 40 years have only rarely encountered cases of chyluria or chylocele. Elephantiasis seems to be absent, and filarial lymphangitis and varicose lymph glands may have been overlooked in the past. One other case of chyluria was seen in a Filipino this year by Dr. Bartels, but the patient left for the provinces before his blood could be examined. Several physicians say they have seen cases of filariasis at Iloilo.

It seems that the disease may be imported into localities where the conditions for its transmission are apparently unfavorable. Our patient has been living with four other Japanese for four, three, three, and two years respectively. An examination of these four and of a number of other Japanese living in their neighborhood was made late at night with negative results. This is rather surprising when one considers that *Culex fatigans*, the mosquito which acts as a favorable intermediate host in many parts of the world, is one of the commonest species of mosquito found in Manila. However, it should be remembered that, notwithstanding the existence of some very strong presumptive evidence, the exact manner in which filariasis is transmitted is still an open question. The brilliant observations of Manson and Bancroft, showing the metamorphosis of *Filaria nocturna* in the bodies of certain mosquitoes, and the further confirmation and extension of their views by the more recent work of Low[1] and James,[2] all tend to convince one that the disease is transmitted by the bite of certain mosquitoes.* But the facts that a number of persons can live for years with a filariated patient, when apparently a favorable intermediate host is present throughout the

[1] *Brit. Med. Jour.*, June 16, 1900, 1, p. 1456.

[2] *Ibid.*, Sept. 1, 1900, 2, p. 533.

*In the older literature on the transmission of filariasis, *culex ciliaris* was named as the intermediate host, but in recent years our knowledge of the *Culicidae* has been greatly extended, and it has been shown by Theobald (monograph "Culicidae," 1901, 2, p. 136) that *C. ciliaris* is identical with *C. pipiens*, and further that in all probability Manson's original work in China and Bancroft's later work in Australia was not done on *C. pipiens*, but on *C. fatigans* (*ibid.*, 1903, 3, p. 226). This widespread species is a voracious night feeder and occurs in large numbers in Manila. According to Low (*Brit. Med. Jour.*, June 1, 1901, 1, p. 1336), "it is the chief spreader of filarial disease in the West Indies, acting as an intermediate host for *F. nocturna*." "It is inefficient for *F. demarquaii*." That the intermediate host is not restricted to one genus or species of mosquito is shown by the feeding experiments of James, in which *Anopheles rosii* and possibly two species of Culex—*Culex microanulatus* and *Culex albopictus*—were shown to be suited for the metamorphosis of *Filaria nocturna*.

year, without their acquiring the disease, as in our case, and the similar cases cited by Maitland,[1] and the "relative immunity" of Europeans and others who are careful with regard to their food and drink, raise the old question whether Manson may not have been correct in his original assumption that the filaria escape from the mosquito to some watery medium and then gain entrance to their definitive host.

DESCRIPTION OF PLATES.

Plate 13.

(Photomicrographs by Charles Martin, photographer, Bureau of Government Laboratories.)

Fig. 1.—*Filaria nocturna*, × about 390 (double exposure). Showing the general morphology and the viscus-like organ at the junction of the middle and posterior thirds of the parasite.

Fig. 2.—Head end of *Filaria nocturna*, × about 880. The sheath, the three duct-like threads connecting the anterior end of the viscus-like organ with the head end of the embryo, and the transverse striations of the musculo-cutaneous layer may be seen.

Plate 14.

(Figures redrawn by T. Espinosa from original drawings.)

Fig. A.—Represents a dead filaria, showing granular degeneration.

Fig. B.—Drawn from a filaria just before granular degeneration set in. Proportions about correct as seen with the Zeiss $\frac{1}{12}$ oil im., comp. oc. 8. Length, 0.330 mm.; breadth, 0.00765 mm. The distance between the anatomical markings were as follows: A–B, 97.92 μ; B–C, 53.55 μ; C–D, 61.20 μ; D–E, 64.26 μ; E–F, 53.55 μ; total, 330.48 μ, or 0.33 mm.

Fig. C.—Head end of filaria, showing retracted lips and spicule.

[1] *Brit. Med. Jour.*, Sept. 1, 1900, 2, p. 537.

PLATE 13.

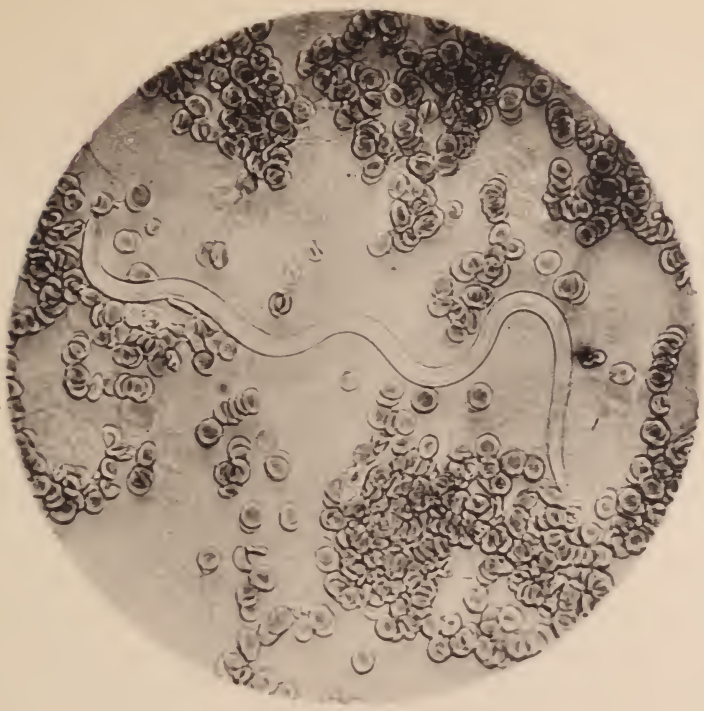


FIG. 1.

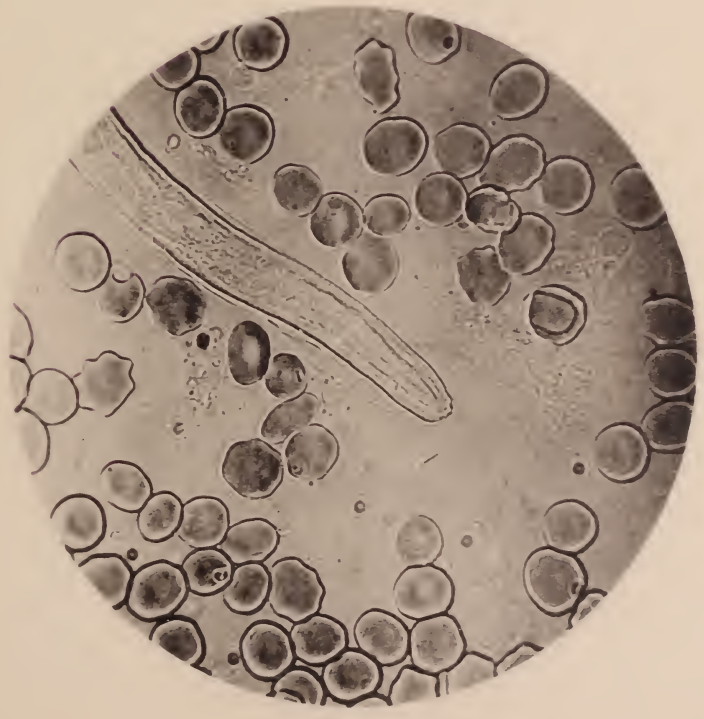
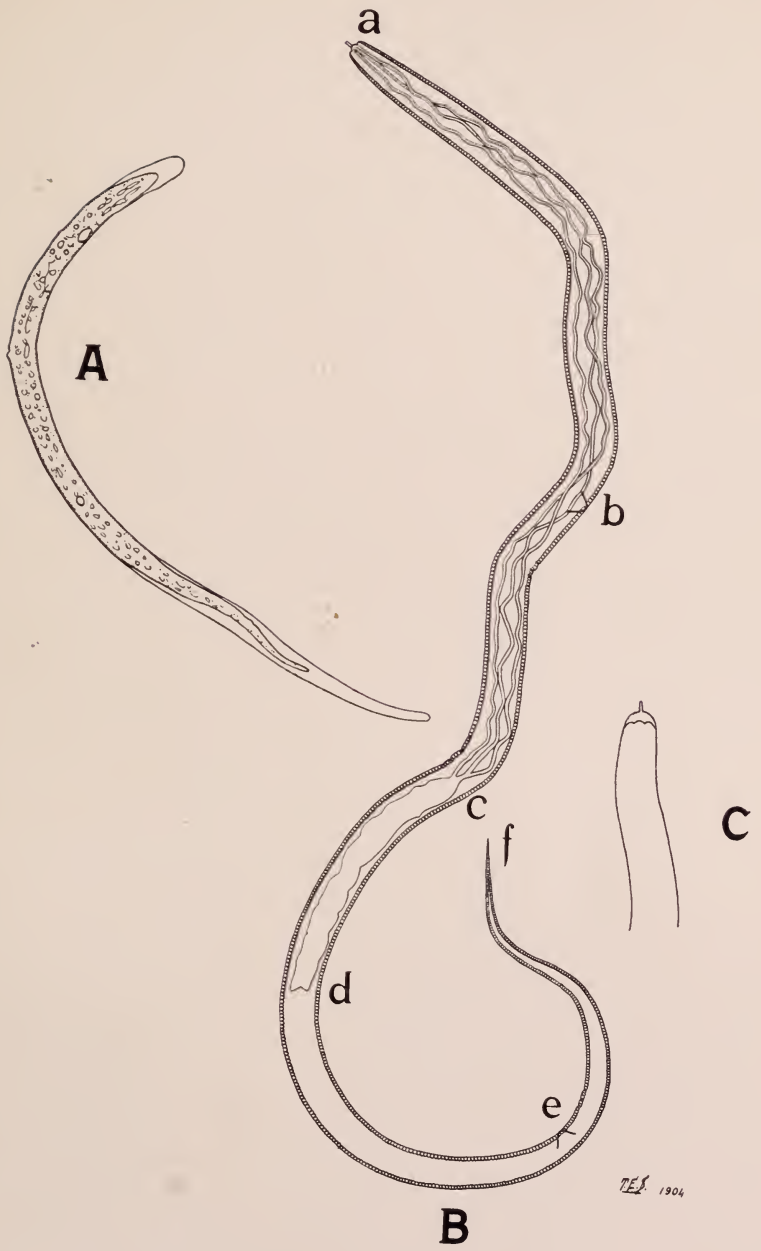


FIG. 2.

PLATE 14.



A CASE OF PULMONARY INFECTION WITH AN ACIDFAST ACTINOMYCES.*

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THE number of interesting cases reported under the names of *pseudotuberculosis cladothrichica*, *pseudotuberculosis streptothrichica*, *actinomycosis atypica pseudotuberculosa*, together with the attempts of Gasperini, Levy and his pupils, Lachner-Sandoval and Neukirch, to establish the botanical position of the so-called streptothrices on morphological and biological grounds, justifies the report of another similar case. It must be stated in the beginning that owing to the absence of cultures and animal inoculations exact species identification is impossible, yet from the morphology and tinctorial peculiarities, as well as the nature of the lesions produced by the organism, it is highly probable that we have to deal with a pathogenic actinomyces similar to the ones described by Eppinger, Aoyama and Miyamoto, W. G. MacCallum, Schabad, and others.

CLINICAL HISTORY.

J. C., white male, 19 years of age, was admitted to the Garfield Memorial Hospital, Washington, D. C., in 1901. The clinical diagnosis was diabetes mellitus. The patient was under observation in the hospital for the greater part of the time, although he returned occasionally to his parents, sometimes remaining with them for a month or two. There was very little change in his general condition from his first admission in 1901 to within a few weeks of his death in March 1903. He was extremely emaciated, very feeble, and presented the classical symptoms of diabetes mellitus in the young. During the last six months of his life he complained of pain in the left infraclavicular region. Physical examination of the chest made at intervals by Doctors Claytor, Deale, Cook, and Morgan revealed puerile breathing over both lungs with more harsh breath sounds over the left bronchus. Patient rarely coughed and examination of the scanty sputum three months before death was negative for tubercle bacilli.

Blood.—Average red corpuscle count 4,580,000 per c.mm. Hemoglobin 80 per cent. Leucocytes ranged between 7,500 and 12,000 per c.mm. Differential counts disclosed a slight lymphocytosis. Bremer's reaction obtained. Fat not demonstrable with osmic acid.

* Received for publication May 2, 1905.

Urine.—Quantity in 24 hours, 2,500 to 3,500 c.c., clear and pale straw in color; reaction, acid; specific gravity, 1035 to 1040; glucose, 3.5 per cent to 8.0 per cent in samples from urine collected in 24 hours. Acetone and diacetic acid present (November 1901 to March 1903). A faint trace of albumen and hyaline cylinders were frequently found.

The amount of sugar eliminated, the presence of acetone and diacetic acid were not appreciably influenced by rigid diet or medication (codeine, arsenic, etc.). On the evening of March 14, patient became delirious, the delirium passed into coma during which there was a marked odor of acetone upon the breath, and death occurred at 5 A. M., March 15.

AUTOPSY.

The autopsy was held four hours after death.

Body of slender build and extremely emaciated. Rigor absent. Numerous hypostases. Skin dry and glossy. Abdomen distended. Subcutaneous fat scanty and pale yellow; abdominal muscles thin. Peritoneal cavity contains the usual amount of clear serous fluid; layers of peritoneum smooth and glistening. Transverse colon prolapsed.

Costal cartilages and ribs soft and flexible.

Pleurae present no adhesions and cavities contain no excess of fluid.

Right lung.—An area of consolidation about the size of a hen's egg is found in the upper lobe. The pleura over this area is dry and lustreless. On section the consolidated portion is grayish yellow in color with scattered whitish opaque nodules. There are central disintegration and softening, with the formation of a small cavity of irregular outline and with shaggy walls, which contains a thin, odorless, grayish-yellow, opaque, pus-like material. The remainder of the lung is pale grayish white, crepitant, with a rather moist cut surface. Well marked carbon deposit.

Left lung.—Crepitant throughout, slightly edematous at base.

Pericardium.—Smooth and glistening; about five c.c. of clear fluid in cavity.

Heart.—Small. Weight 287 gm. Subserous fat abundant. Right side of heart is filled with mixed clot, which is continued into pulmonary artery as a simple red clot. Fluid blood in left heart. Left ventricle wall measures 1.2 cm. Myocardium pale. Mitral valve presents a marked thickening and retraction of posterior leaflet, while on the anterior leaflet there is a yellow, opaque, raised patch about six mm. in diameter. One of the tricuspid flaps presents a similar patch. Aortic and pulmonary valves are normal. Coronaries patulous. Aorta contains numerous yellow, opaque, slightly raised patches of sinuous outline.

Spleen.—Slightly enlarged. Weighs 210 gm. On section the Malpighian bodies and trabeculae are easily visible. Pulp is dark red, very little adhering to the knife.

Kidneys.—Moderately enlarged. Perirenal fat abundant. Right weighs 230 gm.; left weighs 240 gm. Stellate veins injected. Surface smooth. Sections with moderate resistance. Cortex seven mm., vessels prominent, labyrinth of mottled pale yellow hue. Pyramids, purple red. Capsule strips easily.

Liver.—Uniformly enlarged and weighs 2750 gm. Surface smooth and mottled with large areas of a deep yellow color. Section: consistence not increased; similar yellow patches seen on cut surface. Gall bladder distended with brownish yellow bile: biliary channels patulous.

Stomach.—Enlarged and distended. Colon enormously enlarged and slightly thickened. Caliber of small intestines equal to that of normal adult colon. Mesenteric glands enlarged, white, and soft.

Pancreas.—Small, soft, and flabby. In the substance of the tail there is a soft hemorrhagic area, 1.5 cm. in diameter. The parenchyma is moderately atrophic. Interstitial tissue not increased. Weight 67 gm.

Bladder distended, containing 1056 c.c. of urine. Wall extremely thin.

Brain and membranes.—Excess of subarachnoid fluid. Blood vessels of pia injected; arachnoid opacities well developed. Substance of brain and medulla everywhere normal in appearance.

Microscopical examination of the pus from the cavity in the right lung.—Smears were fixed by heat, stained with Ziehl's carbolfuchsin heated to the point at which steam was given off and kept there for five minutes, followed with Gabbet's solution. On examining with $\frac{1}{12}$-inch oil immersion, numerous large masses of tangled bright red threads are seen on a background consisting of moderately numerous polymorphonuclear leucocytes, large and small mononuclear cells with sky blue nuclei and colorless or very light blue protoplasm. The individual threads vary in width from two to three or four times the width of the tubercle bacillus; they possess true branches which are given off at right or acute angles. No clubs or bulbous extremities are observed. While the filaments are usually interwoven into a colony of indefinite shape and outline, quite frequently a decided radiate arrangement is demonstrable. The protoplasm of the filaments stains a clear red, but is frequently interrupted by clear spaces, and the free ends of some filaments are almost colorless. On the other hand a tinge of blue is almost never found in any of the filaments. The colonies are so large that they can be seen easily with a $\frac{3}{2}$-inch objective, and the beading of the filaments is so clean cut that it can be seen without any effort through a $\frac{1}{6}$-inch lens.

HISTOLOGICAL EXAMINATION.

Brain and medulla apparently normal. There are numerous small and large vacuoles in the epithelial cells of the kidney and liver: otherwise these organs are normal. Mesenteric lymph nodes hyperplastic. Slightly enlarged Malpighian bodies in the spleen. The pancreas shows marked atrophy of the glandular lobules, but no appreciable changes in the epithelial cells. Interlobular fibrous tissue not excessive. The islands of Langerhans are apparently normal in size, number, and architecture. Sclerosis and hyaline degeneration are wholly absent.

Wall of cavity in right lung.—On examining a large section stained with hematoxylin and eosin and taken from the lung surrounding the cavity, there are seen with the naked eye deep blue roughly circular areas, about four mm. in diameter, surrounded by a zone of bright pink airless tissue. Around this the lung is porous and of a more delicate pink hue. The blue areas correspond to collections of cells, largely polymorphonuclear leucocytes, among

which a few alveoli plugged with fibrin are seen. In general the alveolar outline is indistinct, yet in sections treated by Weigert's method for elastic tissue the alveolar scaffolding is present, although very greatly fragmented. While many of the polymorphous nuclei are well preserved, there is a marked tendency to karyorrhexis, and fine chromatin droplets are sprinkled throughout the nodule. Larger cells with more faintly staining, single nuclei and a thin rim of pink protoplasm are not infrequent. By the confluence of such areas, large irregular patches measuring 1.5 cm. to 2 cm. are formed in which groups of aveoli filled with fibrin and leucocytes alternate with large dense collections of polymorphonuclear leucocytes. In sections stained by Weigert's fibrin method numerous tangled masses of branched and sometimes beaded organisms are found in the cellular areas. In fact, wherever a dense collection of polymorphonuclear cells is found one can almost invariably demonstrate the delicate filaments of the actinomyces. Other organisms are not present. Numerous attempts to stain the organisms by methods employed for the tubercle bacillus were unsuccessful and genuine tubercle bacilli were absent. Controls made at the same time with the tuberculous tissues in the laboratory offered no especial difficulty in staining or finding the bacilli.

In the consolidated lung surrounding the nodules the fibrinous exudate is very abundant. Some alveoli contain chiefly fibrin in which a few leucocytes and large mononuclear cells are entangled. Many contain moderately numerous leucocytes and few mononuclear cells intermingled with less fibrin, and in a few alveoli leucocytes predominate, only a few small fragments of fibrin remaining. Strands of fibrin passing from one alveolus to another through the pores of Kohn are frequently encountered. There is no trace of organization of the exudate.

In the adjacent air-containing tissue, the alveoli are partially filled with a finely granular, sometimes homogeneous, albuminous material, a few polymorphous leucocytes, and a varying number of large mononuclear cells. The albumen stains light pink with eosin and does not stain by Weigert's method for fibrin. The leucocytes are fairly numerous in some alveoli, very scarce in others. The mononuclear cells present a great diversity of appearances. Some are clearly derived from the alveolar epithelium, for in many alveoli the lining epithelium is distinctly cuboidal or polygonal in outline, and similar polygonal cells possessing a nucleus fairly rich in chromatin surrounded by a moderate amount of pink protoplasm with just a suggestion of violet are found lying free near the center of the alveolus. Others have a smaller, eccentric, deep blue nucleus and clear, relatively abundant, almost colorless, protoplasm. A third variety is represented by a much larger cell with a large vesicular nucleus, frequently situated at one side and surrounded by abundant, faintly staining protoplasm, which contains a few or sometimes many small round brownish black pigment masses. Potassium ferrocyanide followed by hydrochloric acid has no effect upon this pigment. Occasionally large cells with two or three nuclei are found, but Langhans' giant cells are never encountered. Except when bounded by an interlobular septum, there is no sharp demarcation of the nodular consolidated areas from the edematous air-containing lung, the leucocytic infiltration passing gradually into the fibrinous zone, and the latter insensibly giving place to inflammatory edema.

The small bronchi and the bronchioles are the seat of intense inflammation. Desquamated epithelial cells, leucocytes, and fibrin occupy the lumen. The bronchioles are usually plugged with leucocytes and frequently only vestiges of a bronchiole remain—a strip of columnar epithelium and a few muscle fibers embedded in collections of leucocytes. The filaments of the actinomyces are usually present within these structures, the number of colonies bearing a direct relation to the amount of destruction in the bronchial walls. While it is not possible to demonstrate bronchioles within many of the nodules composed of leucocytes, the occasional presence of a row of epithelial cells and remnants of muscle fibers together with the intimate relation to the colonies of the actinomyces reveals the bronchogenic nature of the process.

The alveolar capillaries contain an excess of polymorphonuclear leucocytes but are not engorged with red corpuscles. In the areas of leucocytic infiltration and fibrinous exudation, many of the capillaries are filled with hyaline thrombi. Save for the presence of an increased number of leucocytes, small amount of fibrin, and an adventitial mantle of small mononuclear cells, the vessels of medium and large caliber present no striking changes. Filaments of actinomyces are not demonstrable in any of the vessels.

The lymphatic vessels in the perivascular and peribronchial tissues and in the interlobular septa are markedly dilated and contain numerous polymorphonuclear leucocytes, large and small mononuclear cells with varying quantities of fibrin. The actinomyces is not present.

Although the interlobular septa and the alveolar walls are much thickened, careful staining after the methods of Van Gieson and Mallory fails to show any increase in the fibrillar connective tissue. The septa are widened by the presence of fluid and fibrin, and by the dilated lymphatics. The apparent thickening of the alveolar walls is due solely to the distended capillaries.

On the pleural surface there is a thin fibrinous exudate. The subpleural connective tissue is edematous. There is very slight carbon deposit.

Sections taken from other portions of the right lung and from the left lung show a slight degree of edema, but are otherwise normal, no organisms being demonstrable. The bronchial glands contain a small amount of carbon. Neither the actinomyces nor the tubercle bacillus can be found in them.

*Distribution of the organisms.**—The filaments of the actinomyces are found only in the pus in the cavity, and in the bronchopneumonic tissue constituting the walls of the cavity. In the latter the organism is present in the small bronchi, in the bronchioles, and in the air-sacs filled with leucocytes. In the alveoli containing fibrin or serous fluid the filaments are rarely found, and in the blood vessels and lymphatics they are never seen. The best method of demonstrating them in the tissues in this case is some modification of Gram's method, preferably Weigert's. Concerning the morphology of the fungus in the tissues, there is no deviation from its appearance in the pus. Tangled masses of branching threads, sometimes in a distinct radiate

* As far as we know neither the pus nor the tissues contained any organisms besides the actinomyces. For this reason the tissue changes can hardly be ascribed to mixed infection.

arrangement with beaded filaments in the more central portions are the usual forms encountered. In a painstaking examination of hundreds of such colonies no clubbed forms were observed. The fragmentation or plasmolysis is never so extreme as to suggest micrococci. Sections stained with the usual nuclear and combination stains, as well as with methylene blue, bismarck brown, sudan III, safranin, or the various methods for the tubercle bacillus give no clue to the presence of the actinomyces.

SUMMARY.

From the foregoing description it will be seen that the pathogenesis of the process is about as follows: entrance of the actinomyces into the respiratory passages and lodgment in the bronchioles where an active emigration of leucocytes into the lumen of bronchioles and especially into the adjacent alveoli is incited; farther removed from the organisms the inflammation is less intense and is expressed by a fibrinous exudate; still less is the stimulus felt in the more distant portions of the lung and an inflammatory edema results; by the confluence of the nodules composed largely of leucocytes, and subsequent softening of the resulting mass, cavity formation ensues. This coincides in essentials with Ribbert's investigations and views on bronchopneumonia in general.

COMPARISON WITH SOME SIMILAR CASES.

While information as to the biological characters of this organism is wanting, the close agreement in morphology and staining properties with the organisms of Aoyama and Miyamoto, Schabad, and others, and especially the similarity in the lesions make the relationship or even identity highly probable. A peculiarity in the acid-resisting properties of this organism is the failure to demonstrate it in sections of the hardened tissue by methods designed for the tubercle bacillus. This is entirely in accord with the experience of Berestnew, working with *Actinomyces asteroides Eppingeri*, *Actinomyces farcinica*, as well as with an acidfast actinomyces isolated by him. Flexner and Schabad record parallel experiences.

AS TO NOMENCLATURE.

Savaugeau and Radais have shown that the word "streptothrix" was first used by Corda, in 1839, for a mould wholly

different from the organisms now designated as streptothrices. Having this in mind Gasperini and Lachner-Sandoval have urged the adoption of the group name "actinomycetes" (actinomyces, Harz 1877) on the grounds of priority. Lachner-Sandoval also pointed out that the true *Oospora* differ essentially from the actinomycetes, and, therefore, the latter cannot be included in the genus "oospora." It is hardly necessary to consider the designation "Nocardia," since the permanent adoption of a man's name for a group of microorganisms is without a precedent in the history of bacteriology. There is certainly little desirable in a nomenclature which is historically inaccurate, etymologically meaningless, and of no descriptive value. Actinomyces, consequently, has priority in its favor, and it is also to be noted that it is etymologically preferable since the radiate development of the colonies is a constant and striking feature. Schabad, following Berestnew, erects a subgroup *Actinomyces atypica* for those organisms which do not develop clubbed ends and consequently do not form "Drusen;" are not present as sulphur granules in the pus; and which are acidfast. He affixes *pseudotuberculosa* to those which produce a tubercle-like disease in the lower animals (Eppinger, Aoyama and Miyamoto, MacCallum, Schabad, Horst, and Stokes). While "pseudotuberculosa" is descriptive of the pathological changes, and also implies the relation of the organism to the tubercle bacillus, it has already been used for so many diverse processes that its omission would be in the interest of simplicity in terminology. Although in the majority of instances clubs have not been encountered in the study of these particular organisms, MacCallum has shown that even typical "Drusen" may be a part of the life cycle of some members of the group.

While the pathological picture is not a uniform one, in the majority of instances there is an accumlation of grayish pus in a pulmonary cavity of extremely irregular outline, and with shaggy walls. The proliferation of fixed connective tissue cells frequently does not occur (Aoyama, Schabad, Buchholz), and consequently encapsulation by a comparatively smooth, thick, fibrous wall as in the usual form of tuberculosis is absent. It may be said that the gross appearance stands between a nodular broncho-

pneumonia on the one hand and caseous pneumonia on the other, the bronchopneumonic nature of the condition being expressed by the opaque grayish nodules surrounding the pus collection, while the tendency of the nodules to coalesce, soften and break down leaving an excavation with ragged walls and accompanied by fibrinous and gelatinous pneumonia in the contiguous tissue resembles more or less closely the caseous pneumonic type of acute tuberculosis. The absence of true caseation and the failure to find tubercle bacilli are, of course, the distinctive features. There is almost always an accompanying pleurisy of varying degree. The two extremes are probably the empyema encountered by Birt and Leishman, and the slight dryness and opacity of a small area of the visceral layer in the case here reported.

Turning for a moment to the historically older form of actinomyces, we find a miliary "pseudotuberculosis" caused by a club-bearing actinomyces, described by Pflug in 1882. In Boström's classical work on human actinomycosis reference is made to an autopsy on a man dead of phthisis, at which pneumothorax, pleurisy, and a small cavity in the right lung were found. The cavity was lined by a necrotic villous wall and contained a gray-red turbid fluid. In this fluid and in the necrotic tags of tissue projecting into the excavation grayish white granules were present. On microscopical examination the granules were seen to be composed of densely packed, long, usually spiral, genuinely branched filaments of radial arrangement and without thickening of the free ends. Boström regarded this as a very recent invasion of the lung by an actinomyces, and yet many such cases have been reported in following years under the head of pulmonary streptothricosis. Orth in his textbook, describing pulmonary actinomycosis and having in mind the organisms of the *Actinomyces bovis* group, gives as characteristics the formation of confluent abscesses with a tendency to involvement of the pleura, vertebral column, pericardium, diaphragm and peritoneum. The majority of these lesions are reproduced by the acidfast actinomycetes and by those which do not under ordinary conditions form clubs. Thus Birt and Leishman found empyema and pericarditis. An excellent example of the burrowing character of the

lesions is found in Schabad's case, where in addition to the pulmonary cavity, there were pleurisy, caries of the adjacent ribs, and an intermuscular abscess. Quite recently Sanfelice in a work on streptothrix-tuberculosis, draws the closest analogy in the biology of certain actinomycetes and the tubercle bacillus, and in the histology of the lesions produced by them. Employing intravenous injection of some air actinomycetes, he produces in dogs cavity formation in the right lung without a generally disseminated "streptothricosis." The histogenesis of the lesions is almost identical with that of true tubercles; giant cells, epithelioid cells, a mantle of wandering cells, and acidfast bacillary forms of the fungus being found on microscopical examination. This is somewhat different from the results obtained by other investigators, many of whom place the process among the inflammatory necrotic conditions rather than among those of the productive or granulation type. In the cases of Aoyama and Miyamoto, Norris and Larkin, and in the present case the lesions were essentially acute inflammatory and necrotic in nature.

There is, therefore, a large group of acidfast actinomycetes without clubbed extremities, many of the individual organisms differing fundamentally in their cultural characters and comparative pathogenesis for the laboratory animals, nevertheless producing similar or almost identical lesions in the human lung. On the other hand two actinomycetes with the closest possible biological relationship may produce widely different pathological pictures. It is needless to refer to the possible variation in the virulence of the organisms and the resistance of the host. The similarity in the pulmonary lesions produced by the many acidfast actinomycetes as well as the imitation of the pathological anatomy and course of the conditions due to the ordinary club-bearing varieties is certainly more than a coincidence.

The interest in this group of cases lies in the extremely wide geographical distribution and in the possibility of diagnosis during life (Lubimow, Birt and Leishman, Aoyama and Miyamoto, Schabad, and Warthin). The case in hand claims attention in being an exception to the rule that all diabetic phthisis is tuberculous. To what extent the glycemia predisposed to or affected

the course of the disease is not known. At the same time the especially luxuriant growth of the pathogenic actinomycetes in media containing two per cent of glucose is very suggestive.

Finally, it remains for me to express my indebtedness to Dr. J. B. Nichols for the control of the material, and especially to thank Dr. MacCallum for stimulating and guiding my interest in this group of cases.

REFERENCES TO RECENT CASES.

(The literature can be collected so easily from the writings of Schabad and Horst, that it is only necessary to mention new works bearing upon the subject.)

HORST. "Ein Fall von Streptothrixpyaemie beim Menschen," *Ztschr. f. Heilk*; Abtheil. f. path. Anat., 1903, 24, p. 157.

SCHABAD, J. A. "Actinomycosis atypica pseudotuberculosa," *Ztschr. f. Hyg. u. Infectionskrankh.*, 1904, 47, p. 41.

WARTHIN AND OLNEY. "Pulmonary streptothricosis," *Amer. Jour. of the Med. Sci.*, 1904, 128, p. 637.

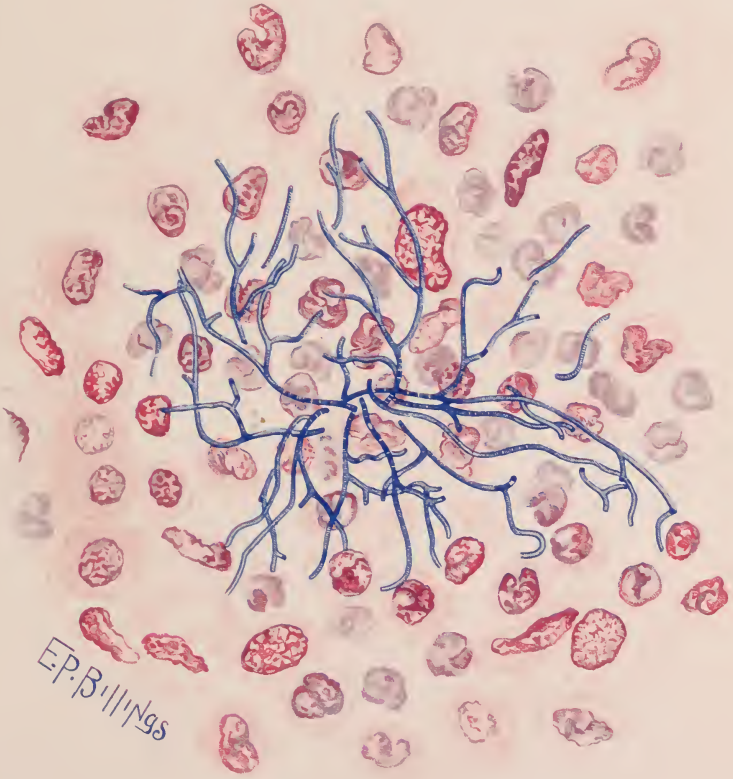
STOKES. "A Study of the Group Actinomyces," *Amer. Jour. Med. Sci.*, 1904, 128, p. 861.

SANFELICE. "Streptothrix-Pseudotuberkulose," *Centralbl. f. Bakt.*, 1905, 38, p. 30.

EXPLANATION OF PLATE 15.

A young colony of the organism from one of the bronchopneumonic nodules. The radiation, branching, and slight beading of the filaments is clearly shown. The predominating cells are polymorphonuclear leukocytes; large mononuclear cells are figured, and to the left there is a row of alveolar epithelial cells. Section is stained with carmine and Weigert's method for fibrin. Magnification about 1500.

PLATE 15.



BACILLUS MYCOGENES (BACTERIUM MUCOGENUM), NOV. SPEC. AN ORGANISM BELONGING TO THE BACILLUS MUCOSUS CAPSULATUS GROUP.*

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THE place of members of the group of the bacillus mucosus capsulatus in human pathology, and their etiological importance in the production of inflammatory conditions, has been emphasized so much in the past few years, that it is needless to review in any detail, the literature of the subject.

The recent articles of Friche, Howard, Clairmont, Strong, Blumer, Mallory and Wright, and a number of others, have served to elucidate some of the problems presented by the description of a large number of capsulated bacteria closely related to the organisms originally described by Escherich, Friedländer and Pfeiffer. The most recent and most comprehensive work upon these organisms is that of Perkins[1] who had exceptional opportunities for studying these bacteria, in consequence of their frequent appearance in the autopsies in Cleveland. From Perkins' investigation, it is apparent that, among the large number of capsulated organisms described as distinct species, but three main types can be established.

All the organisms, thus far described, are fairly large non-flagellated bacteria, surrounded by a mucilaginous capsule; they grow upon the surface of media, as abundant, slimy, elevated, discrete or confluent colonies and fail to secrete any proteolytic enzyme. The different organisms differ much, however, in their action upon carbohydrates.

The majority of the species are capable of fermenting all the known carbohydrates, (including sugars, some of the starches and some of the alcohols), with the production of acid and the evolution of gas made up of a mixture of hydrogen and carbon dioxid.

*Received for publication February 20, 1905.

[1] *Jour. Infect. Dis.*, 1904, 1, p. 241.

These species all acidify and coagulate milk, and are identical with the bacillus lactis aërogenes described by Escherich.

Certain species fail to ferment lactose, and because of inability to split up this sugar, fail also to coagulate milk. Such organisms are identical with the bacterium pneumonicum of Friedländer, usually known as Friedländer's bacillus. The fact that Friedländer's bacillus never coagulates milk has been overlooked by a number of observers, although this characteristic was pointed out as early as 1886 by Fluegge.[1]

Other organisms retain the capacity to coagulate milk and ferment lactose, but exert no fermentative action upon saccharose. They thus bear to the organisms fermenting all the carbohydrates, the same relation which the non-saccharose fermenting variety of the bacillus coli bears to the variety that acts on this sugar. The bacteria of this type are all identical with the bacillus acidi lactici of Hueppe, with which also is probably identical the bacterium duodenale described by Ford,[2] in the upper portion of the intestinal tract.

A number of other species have been described from time to time, with somewhat different cultural characters. The most important of these is the rhinoscleroma bacillus, whose identity is not firmly established either as a distinct species or as the etiological factor in rhinoscleroma. Apparently two capsulated organisms have been described in this connection. They have in common, growth in milk without coagulating it, but differ in their action on the sugars. Some species ferment dextrose and saccharose and are thus identical with the Friedländer bacillus, while others have no action upon the carbohydrates. Whether the latter microorganisms represent distinct types belonging to this group or whether they are degenerate forms of organisms originally possessing the capacity to break down the carbohydrates, has not yet been settled definitely. Perkins inclines to the opinion that the non-fermenting types are derived from organisms originally capable of attacking the sugars, and which in the course of adaptation to saprophytic existence have lost this character. However this may be, in no

[1] *Die Mikroorganismen*, 1886.

[2] *Studies from the Royal Victoria Hospital Reports*, 1903, 1.

case have organisms of this type been found in definite association with pathological changes in man.

A recent series of local infections in the wards of the Johns Hopkins Hospital, the exudate of which was subjected to bacteriological examination, and from all of which the same microorganism was obtained, possessing constant cultural features and marked pathogenic action upon the smaller animals, makes it apparent that certain non-fermenting microorganisms must be included in the group of the bacillus mucosus capsulatus and that these microorganisms in no sense may be looked upon as degenerate forms.

Case 1.—M. B., a girl, about 22 years old, was admitted to the surgical wards with pain in the right knee. For three years she had had some weakness in that knee, which cracked when under a strain, but 19 months previous she had fallen and bruised her knee, considerable ecchymosis resulting. There was nothing in her history to suggest gonorrhea and the chronicity of the case was the only thing to suggest tuberculosis. Upon examination there was found a flexion of the knee to about 15° with a further possible flexion of about 45°, an increase of fluid in the joint and some atrophy of the muscles above and below the joint.

The joint was opened and found to contain considerable clear fluid with a few flakes of fibrin and a slightly reddened synovial membrane, from which projected a few villi. The villi were removed, the joint washed out with salt solution, olive oil injected, and the joint closed. The wound healed rapidly, but there remained, however, some limitation of motion.

The excised villi were hardened and stained for bacteria and the fluid examined culturally. In the excised villi were found short oval bacteria, which retained Gram's stain and which were surrounded by a capsule. From the exudate grew but one species, and this was recognized as a probable member of the bacillus mucosus capsulatus group. Its major characters were identical with those of the other members of the group, but some striking differences were noted.

Morphologically, the organism is a short, plump bacterium, non-flagellated, non-spore-bearing, usually measuring less than 1 μ in its smaller diameter. Capsules can readily be demonstrated by Welch's method of precipitating the mucin of the capsule by glacial acetic acid and then applying gentian violet. They can be obtained most easily from the exudate of an inoculated animal, but may be seen in milk cultures and rarely upon the surface of agar-agar. The organism occurs singly or in pairs, depending upon the age of the culture, and may develop in short, or even in long chains of 10 to 15 elements. It retains Gram's stain in the tissues, but, as a general rule, may be decolorized in smears from cultures, especially if the alcohol be left on the film for some time.

Upon the surface of agar-agar, it grows as a porcelain-white, spreading mass of great viscosity. In broth and in milk this character is accentuated, the platinum loop drawing up strings of mucus-like material several inches

long. Its colonies on agar-agar and on gelatin are smooth and elevated and when lightly seeded have the dew-drop or mucoid appearance characteristic of the capsulated bacteria. The organism fails to liquefy gelatin and blood-serum, and in stab culture exhibits the "nail-head" growth. It acidifies and coagulates milk in from one to five days, at the same time reducing the litmus, but no digestion of the casein occurs. Upon potato, it grows as a dark-brown slimy mass, but no gas blebs are formed either upon the surface of the potato or in the surrounding fluid. It does not form indol.

In these reactions the organism is identical with the main types of the group. Upon the employment of carbohydrates, however, either as a constituent of nutrient agar, or in combination with sugar-free broth in fermentation tubes, no gas formation was noted with any carbohydrate. The fluid in the closed arm of the fermentation tube always remained clear, the growth being limited to the bulb, where the reaction was always alkaline. As we have seen there is acid formed in milk; and on smears of the sugar agars, there is acid formation with lactose early, with glucose after several days; and some of the other sugars show acid reaction after standing a long time, but stabs do not show acid formation except at the surface and that after some time. Thus we see that while under certain conditions there is action upon lactose, if oxygen is present, there is in the ordinary sense of the word, no fermentation. Dextrose, saccharose, lactose, mannite and glycerin were tested and none of them were fermented by this organism.

Pathogenic action.—When freshly isolated, this organism was highly virulent for rabbits and guinea pigs: 1/100 c.c. of a 24-hour broth culture, injected subcutaneously killed a rabbit in 18 hours. The animal presented a typical picture of a bacteriemia, the organism being recovered from the heart's blood and from the internal organs. The recovered organism was similar to the organism injected, but all its cultures were more viscous. The virulence of this culture for rabbits was subsequently much lessened, as a month later, a rabbit survived a dose of two c.c. Guinea pigs were also very susceptible, and for them the virulence was great at the end of two months. They presented in all cases the picture of bacteriemia and one case which lived longer than the average, 15 hours, had a well developed peritonitis, but cultures showed only the presence of the organism injected. The organism recovered from all the animals was tested carefully with carbohydrates and in all cases the reactions were like those of the original culture.

Case 2.—W. H., colored, male, age about 30 years, was injured in a railway accident, "two cars ran over both legs". He was admitted to the hospital a few hours after the accident and the right foot was amputated and a plaster cast applied to the left foot, which was crushed without any lesion of the skin. The amputated stump healed readily, but the left foot became gangrenous, the gangrene gradually extending up the leg. There was no subcutaneous emphysema. The left leg was then amputated just above the edge of the gangrene. Upon incising the gangrenous part gas with a peculiar scorched odor and which burned when lighted, escaped. Both aërobic and anaërobic cultures were made from the gangrenous mass. The patient made a slow, but satisfactory recovery from the second operation.

Upon the anaërobic cultures an organism was obtained which was probably identical with the gas bacillus of Welch. From the aërobic cultures an

organism was obtained which proved identical with the organism obtained from the previous case. It was also pathogenic to smaller animals, mice, guinea pigs and rabbits dying from inoculation with small amounts, within 15 hours.

Case 3.—P. T., colored, female, aged 20, was shot in the mouth, six weeks before admission to the hospital. The wound made by the bullet failed to heal, and there had been a constant discharge of pus and blood since the accident.

At operation a large abscess was found just behind the angle of the jaw on the right side and quite near the great vessels. The abscess was evacuated and some necrotic bone removed, but the bullet was not found. The wound did not heal well, and following the closure of the sinus, another abscess formed in about the same place. The wound was again opened and the pus evacuated, the bullet being found, and the wound allowed to heal by granulation. Recovery followed.

Cultures made from the pus at the second operation revealed a single microorganism, which was carefully studied and found to be identical with the one described in Case 1. It was pathogenic for mice and guinea pigs. It was not virulent for rabbits when tested some months after it was first isolated at a time when it might have lost its virulence for rabbits, as the other cultures did, in that length of time.

This organism has been kept in stock in the bacteriological laboratory of the Johns Hopkins Hospital since its original isolation and when tested recently, nearly a year after its original isolation, was found to give the same cultural reactions.

We thus have a species of microorganism obtained upon three different occasions, which is capable of causing mild inflammations especially of serous membranes in man, and is possessed of considerable virulence for smaller animals. It is furthermore characterized by certain fundamental peculiarities which place it in the group of the bacillus mucosus capsulatus, but differs from the other members of this group in its failure to ferment the carbohydrates.

So far as can be learned by examination of the literature, no organisms of this description have thus far been observed in human lesions, and I therefore feel justified in describing it as a new species for which I propose a name: Bacillus mycogenes (Bacterium mucogenum), nov. spec., Edwards, 1905.

In conclusion, it gives me great pleasure to express my thanks to Dr. Halsted for permission to report the cases from his surgical clinic, to Dr. Bloodgood and Dr. Haskell for the opportunities for research in the laboratory of surgical pathology, and to Dr. Ford for his suggestions in regard to the conduct of the bacteriological investigation.

A SEARCH INTO THE NITRATE AND NITRITE CONTENT OF WITTE'S "PEPTONE," WITH SPECIAL REFERENCE TO ITS INFLUENCE ON THE DEMONSTRATION OF THE INDOL AND CHOLERA RED REACTIONS.*

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IN a previous publication[1] I concluded, by rather rough experimental methods and deductions, that the cholera spirillum is not a nitrifying organism and that the successful demonstration of the cholera red reaction in a culture grown in a solution of Witte's "peptone" depends upon the reduction of a trace of nitrates. I was unaware at the time my experiments were performed that Petri[2] and Bleisch[3] had already performed quite extensive and conclusive experiments upon this point. The latter worker also pointed out that the presence of an excess of nitrates or nitrites in the medium interfered with the reaction, and that the nitrate content of ordinary broth is so inconstant as to make it valueless for diagnostic purposes.

Some peculiar results in testing for indol, during the study of some bacteria from a case of irregular fever, emphasized for me the urgent necessity of adopting methods of preparing and testing media which would indicate the presence or absence of certain chemical constituents influencing such a biochemical test. This led to an investigation into the manner in which nitrates and nitrites may gain an entrance to media, and the influence of such variations on the demonstration of the indol and cholera red reactions.

It does not seem to me that the distinct difference between the indol and cholera red reactions has been sufficiently empha-

* Received for publication April 10, 1905.

1 Bulletin No. 19, 1904, *Bureau of Government Laboratories*, Manila, P. I.

2 *Centralbl. f. Bakt.*, 1889, 5, 561.

3 *Zeitschr. f. Hyg. u. Infektionskh.*, 1893, 14, 103.

sized in the past. By reference to the above-mentioned publication it will be seen that I did not fully appreciate this difference myself, for reasons which will be evident. Salkowski and Nencki[1] mistook the *purple* colored indol reactions for the cholera *red*, and although Petri and Bleisch undoubtedly produced the cholera red reaction, they do not dwell on the difference between it and the indol reaction. Kolle[2] says that "on adding small quantities of concentrated, chemically pure sulphuric or hydrochlorie acid to bouillon or peptone cultures of cholera vibrios a violet or burgundy-wine-red color appears, as was shown by Poehl, and later by Bujwid and Dunham." Now there is a very distinct difference between the violet or purple colored indol reaction and the vermilion colored cholera red reaction when these tests are performed under carefully controlled conditions; and since both tests are of value in the differentiation of species, it seems advisable to define further the conditions under which they must be performed.

Further, my experiments have convinced me that the cholera spirillum does not produce nitrites in Dunham's peptone solution, made from Witte's "peptone" dialyzed free from nitrites, and that the apparent production of nitrites can be explained by the testing of uninoculated controls.

RESULTS OF A SEARCH FOR NITRATES AND NITRITES IN SOME INGREDIENTS USED IN PREPARING MEDIA.*

In testing for nitrogen, as nitrites, the naphthylamine hydrochloric and sulphanilic acid test was used in the manner usually employed in nesslerizing for ammonia, excepting that for purposes of comparison the reactions were performed in culture test tubes. In practice one c.c. of each test solution was added to 10 c.c. of the fluid to be tested. Where nitrates were sought for, the phenol sulphonic acid test was used and the reactions performed in three inch porcelain evaporating dishes.

The preliminary examination of a number of bottles of distilled water furnished by the Government Ice Plant showed that

[1] Vide the article by M. BLEISCH, *loc. cit.*

[2] KOLLE UND WASSERMAN, *Handbuch der Pathogenen Mikroorganismen*, 1903, 3, 21.

*I am indebted to Mr. C. L. Bliss, Physiological Chemist of the Bureau, for valuable advice during the course of the experiments.

this water sometimes contained nitrites in considerable quantities. So water was redistilled from potassium permanganate, collected in a perfectly clean fashion, and tested to prove the absence of nitrites and nitrates before use.

Several samples from a bottle of Kahlbaum's chemically pure sodium chlorid were found free from nitrates and nitrites. This was employed throughout the following experiments.

In testing "peptones" 0.1 gm. was dissolved in 10 c.c. of pure water, thus representing 10 c.c. of a one per cent peptone solution. In this way all our stock of Witte's "peptone," eight bottles, and in addition one bottle of Grübler's Pepton depur. sicc., were tested for nitrites. Some of the tests were repeated more than once, as indicated in Table 1, and in the last analysis, when the reactions were sufficiently marked, they were compared with the standard sodium nitrite colorimetric scale usually employed in the quantitative estimation of nitrites.

TABLE 1.

"Peptone"	No. of bottles	Nitrites (In 0.1 gm. "peptone") (In 10 c.c. water)	Nitrates (In 0.1 gm "peptone"
Witte's Peptonum Siccum	1	(1) trace (2) 0.0000005 gm.N.	0
Witte's Peptonum Siccum ..... Pro Bacteriologie	2	(1) 0 (2) 0 (3) 0	0
Peptonum Siccum	3	(1) distinct reaction (2) 0.0000005 gm.N.	Not tested
Peptonum Siccum	4	(1) distinct reaction (2) trace	Not tested
Peptonum Siccum ..... Pro Bacteriologie	5	(1) trace (2) distinct reaction (3) 0	0
Peptonum Siccum	6	(1) trace (2) trace	Not tested
Peptonum Siccum	7	(1) trace (2) distinct, but less than 0.0000005 gm.N.	Not tested
Peptonum Siccum	8	(1) 0 (2) trace (3) trace	Not tested
Pepton depur. sicc. Grübler.....	9	(1) trace (2) trace	Not tested

These are the only records I have of a considerable number of such tests, but they suffice to show that nitrites occur in some

"peptones" in varying and often considerable quantities. They further show that nitrites may be distributed irregularly throughout the mass of "peptone," for when one sample, for instance, from the surface reacts to the test, another from a deeper portion of the bottle may be free from nitrites, or vice versa. This, together with further evidence to be presented, plainly indicates why the indol reaction may be obtained with one lot of media by the addition of chemically pure sulphuric acid alone, while with another the addition of nitrite is necessary.

All these "peptones" give a strong Biuret reaction, but only one was tested for the presence of peptones. Ten grammes from bottle No. 5 were dissolved in pure water and placed in a parchment dialyzer with all due precautions against introducing nitrites. After six hours at 28° the diffusate (about 150 c.c.) was tested. It did not give the Biuret reaction and 10 c.c. gave a reaction for nitrites equal to 0.0000005 gm.N.

Further, it was necessary to wash filter paper free from nitrites, e. g. a filter paper (Schleicher and Schülls) was washed with 200 c.c. nitrite-free water; 10 c.c. of the filtrate gave a reaction for nitrites corresponding to 0.0000005 gm.N.

The sulphuric acid used throughout these experiments was tested to prove the absence of nitrites.

EXPERIMENTS ON THE DEMONSTRATION OF THE INDOL AND CHOLERA RED REACTIONS IN DUNHAM'S PEPTONE SOLUTION.

In Undialyzed Peptone Solution.—Dunham's peptone solution, containing one per cent Witte's "peptone" (Bottle No. 5) and 0.5 per cent sodium chlorid, in redistilled water, was prepared with special precautions to avoid introducing nitrates and nitrites.*

Half of the medium was distributed in test tubes (10 c.c. per tube) and marked "peptone solution only." To the other half was added 0.01 per cent of a freshly prepared solution of sodium nitrate and this was then distributed in a similar manner. Both sets were autoclaved for half an hour at 120°. Final reaction, 0.5

*A large glass beaker was found to be especially convenient for boiling medium, as one can more easily mark the initial height of the latter and watch the completeness of solution, than when the cooking is performed in an agate-ware boiler. The albumoses do not all go into solution in the presence of 0.5 per cent sodium chlorid.

per cent acid to phenolphthalein. A tube of each of the media tested for nitrites gave a reaction equal to about 0.0000005 gm.N.

(*a*) One tube of each medium was inoculated with cholera 579.* After 18 hours' growth at 36° to 37°, 0.5 c.c. of pure sulphuric acid was added to each culture. The culture marked "peptone solution only" yielded an immediate *purple*-colored indol reaction. The culture marked "peptone solution only + 0.01 per cent $NaNO_3$" did not give an immediate reaction, but in about half an hour developed an intense *red* color (cholera red reaction).

(*b*) That this "peptone solution only" is eminently suitable for the production of indol by *B. coli* 577† is shown by the following experiment.

Three tubes were inoculated each day for eight successive days and the cultures kept at 36° to 37°. Bearing in mind the observation of A. W. Peckham[1], that with different cultures (*coli*) the maximum amount of indol could be decided only by corresponding variations in the proportions of nitrites and acid used, I experimented to see whether a similar variation in the amount of nitrites and acid was necessary to elicit the maximum indol reaction in cultures in the same organism, for example, of two and eight days' growth.

To each series of three tubes, then, one-half, one and two c.c. of a 0.01 per cent sodium nitrite solution and corresponding amounts of pure sulphuric acid were added. Throughout the series the most intense reaction was elicited by using 0.5 c.c. of the test solutions, with the exception of the seven-day old series, where 1 c.c. yielded the best reaction. The c.c. invariably yielded enough nitrous acid to obscure the reaction. It may be of interest to plot the gradual increase of indol as shown in Chart 1, where the reactions of greatest intensity are compared with the colorimetric scale of standard sodium nitrite dilutions.

Throughout the following experiments the test for indol was

* Unfortunately I have been unable to test all the cholera cultures described in Bulletin 19 of this Bureau, owing to the accidental death of nearly all my stock cultures. This strain of culture, 579, was reclaimed by plating Dr. W. E. Musgrave's ameba culture 11524 grown in pure symbiosis with this spirillum.

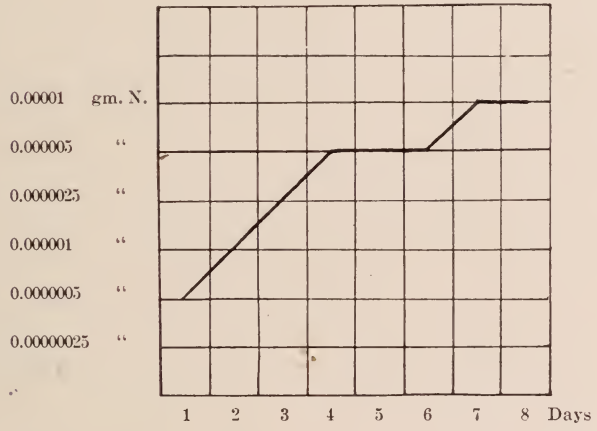
† A culture kept in stock for two years, the fermentation tests of which are given on p. 24, Bulletin 19, *Bureau of Government Laboratories*, Manila, P. I.

[1] *Jour. Exper. Med.*, 1897, 2, 560.

made by adding 0.5 c.c. of a freshly prepared 0.01 per cent solution of sodium nitrite and 0.5 c.c. of pure sulphuric acid. Cholera red was tested for by adding 0.5 c.c. of pure sulphuric acid.

(*c*) Such an albuminous medium, moreover, seems to absorb nitrites from the laboratory atmosphere much more rapidly than distilled water does, for this "peptone" solution, which at first gave reactions for nitrites corresponding to about 0.0000005 gm. N., after two weeks at 18° to 28° gave reactions corresponding to

CHART 1.



about 0.0000025 gm. N. an increase quite sufficient to yield an indol reaction when pure sulphuric acid alone is added to a 24-hour culture of *B. coli* 577.

This increase of nitrites was not due to an increase in the concentration of the medium, for the initial level of the medium had been marked on each tube and very little evaporation had taken place. Further, this increase in the amount of nitrites in the medium containing nitrates is sufficient to obscure somewhat the bright vermilion red color of the cholera red reaction.

In Dialyzed Peptone Solution (Nitrate- and Nitrite-Free).—As there was very little "peptone" left in bottle No. 2, 10 grammes from bottle No. 5 were dissolved by boiling in about 200 c.c. of pure water. It was then dialyzed through parchment with due precautions against introducing nitrates or nitrites or any other

foreign substance. As mentioned above, no peptones were found in the first diffusate (six hour), which gave a reaction for nitrites. The process was continued for 24 hours during two days, the contents of the dialyzer being removed, concentrated by boiling and sterilized at necessary intervals. At this time 10 c.c. of the contents of the dialyzer gave no reaction for nitrites. Dunham's peptone solution was then made up as in the experiments with undialyzed "peptone"—one set being marked "nitrite-free peptone" and the other "nitrite-free peptone + 0.01 per cent $NaNo_3$." After autoclaving, the "nitrite-free peptone" solution gave no reaction for nitrites or nitrates, while the other gave no reaction for nitrites but quite a distinct reaction for nitrates. The final reaction of this dialyzed medium was 0.1 per cent alkaline to phenolphthalein, showing the removal of substances capable of reacting acid to phenolphthalein to the extent of 0.6 per cent.

A series of tubes inoculated with cholera 579 was tested after 17 hours at 36° to 37°, with the following results:

TABLE 2.

Medium	Culture	Hours at 36° to 37°	Nitrites	Cholera Red	Indol	Nitrites in Uninoculated Controls
"Nitrite-free peptone"	579	17	–	–	+ Deep purple color immediately	–
"Nitrate-free peptone" + 0.1% $NaNo_3$	579	17	Intense Reaction	+ Light red color after several hrs. 36° to 37°		–

In about 10 days however, this dialyzed medium had absorbed enough nitrites to give a distinct reaction, and without the use of uninoculated controls one might have assumed that the nitrites were produced through the activity of the cholera spirillum, as may be seen in Table 3.

It will also be noted that the faint trace of nitrites absorbed from the atmosphere was not sufficient to yield any reaction, when sulphuric acid alone was added, but that the addition of nitrite and sulphuric acid produced a very distinct indol reaction.

TABLE 3.

Medium	Culture	Days at 36° to 37°	Nitrites	Nitrites in Uninoculated Controls	Cholera Red	Indol
"Nitrite-free peptone"	Cholera 579	1	Faint reaction	Faint reaction equal to that in tubes inoculated with cholera 579 and of equal intensity when compared with each other	—	+ Deep purple color
"	"	2	All three of equal intensity		—	"
"	"	3			—	"
"	Coli 577	1				—
"	"	2				+ (Faint)
"	"	3				+ (marked reaction)

In the dialyzed medium containing 0.01 per cent sodium nitrate, the cholera spirillum almost completely reduces the nitrates to nitrites in 24 hours, as shown in Table 4.

TABLE 4.

Medium	Culture	Days at 36° to 37°	Nitrites	Nitrates	Cholera Red	Indol
Nitrite-free peptone +0.01% $NaNO_3$	Cholera 579	1	Marked reaction	Faint reaction	Yellowish	Yellowish
"	"	2		Negative	Reddish-yellow	Reddish-yellow
"	"	3		(Not tested)	Distinct and marked *red* color with none of the purple color of indol reaction	Distinct and marked *red* color with none of the purple color of indol reaction
"	Coli 577	1			As per cholera 579, but of lesser intensity	
"	"	2				
"	"	3				

Here an immediate and distinct cholera red reaction was not obtained until the organism had grown for three days at 36° to 37°. This experiment (which is one of a number) also shows that, when growth takes place in the presence of 0.01 per cent sodium nitrate, even if a trace of nitrite be also originally present, something (an oxidation product of indol?) is produced which is demonstrable as "cholera red,"* whether sulphuric acid alone or

*According to Kolle (*loc. cit.*), "cholera red" was isolated in a pure state by Brieger, who considered the cholera red reaction as nothing more than the nitrose-indol reaction, the nitrous acid set free combining with the indol to produce a new substance, the cholera red. Brieger's original articles (*Deutsche med. Wchnschr.* 1887, 13, p. 303) are not within reach, so I cannot discuss this point.

sulphuric acid and nitrite are added subsequently, though such a further addition of nitrite may obscure the reaction.

On the other hand, a few days' growth in the presence of sufficient nitrite to yield the indol reaction does not influence the character of the indol formed; but if a considerable amount of nitrite be present in the medium, the further addition of nitrite yields enough nitrous acid to obscure the indol reaction.

SUMMARY AND CONCLUSIONS.

1. Nitrites, and probably nitrates also, may gain entrance to artificial media from various sources—certain waters, "peptones," and filter papers—yielding distinct reactions for nitrites with naphthylamine hydrochlorid and sulphanilic acid.

2. A sufficient quantity of nitrites may be further absorbed, in a few days, from the laboratory atmosphere, to yield a distinct *indol* reaction upon the addition of 0.5 c.c. of chemically pure sulphuric acid to, for example, a culture of *B. coli* 577 grown for 24 hours at 36° to 37° in 10 c.c. of Dunham's peptone solution, which originally contained insufficient nitrite to yield such a reaction.

3. The cholera spirillum does not produce nitrites in nitrate- and nitrite-free "peptone" solution, prepared from Witte's "peptone" dialyzed free from nitrites; and when nitrites are apparently formed, their presence may be explained by the simultaneous testing of uninoculated controls. In this I disagree with Bleisch, who believed that small quantities of nitrites could be formed, and agree with Petri, who considered the existence of such a nitrifying power as unproved as well as improbable.

4. The vermilion-colored "cholera red reaction" must be distinguished from the purple-colored "indol reaction."

5. The production of "cholera red," by the cholera spirillum or *B. coli*, takes place only, under the conditions of these experiments, during the reduction of a trace of nitrates, and when formed is demonstrable as such, whether pure sulphuric acid alone or sulphuric acid and nitrite are added, although an excess of nitrite furnishes enough nitrous acid to obscure the reaction.

6. The growth of *B. coli* or the cholera spirillum in the presence of sufficient nitrite to yield the indol reaction on the addition of a pure acid alone does not influence the character of the indol formed; but if a considerable amount of nitrite be present in the medium, the further addition of nitrite yields enough nitrous acid to obscure the indol reaction.

7. The cholera red reaction is not specific.

8. It is recommended that media used in testing for the production of indol or cholera red be examined for nitrates and nitrites before use.

THE OCCURRENCE OF FUSIFORM BACILLI AND SPIRILLA IN CONNECTION WITH MORBID PROCESSES.*

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DURING the past three years there have appeared many reports of the finding of bacilli of fusiform shape, often associated with long spirilla, in connection with various pathological conditions. The bacillus has been designated variously as "fusiform bacillus," "spindle-shaped bacillus," "bacillus hastilis," "bacillus fusiformis," "Vincent's bacillus," "Bernheim's bacillus," etc.

According to Tarassiewicz (quoted by Mayer), the first observer to note the association of fusiform bacilli and spirilla with a morbid process was Rauchfus. In 1893 he demonstrated pointed bacilli and spirilla in ulceromembranous angina, and his photographs then appear identical with those described by subsequent writers. Plaut in 1894 described the organisms in five cases of ulcerous angina. Vincent in 1896 described fusiform bacilli and large spirilla in cases of hospital gangrene, and stated that the same organisms were found in certain anginas of an ulcerative type. Bernheim in 1897 reported 30 cases of stomatitis and angina, in all of which he had found fusiform bacilli and spirilla. He appears to have been the first to point out the etiological identity of certain cases of stomatitis and angina. Vincent, in 1898, reported a further series of 14 cases of ulceromembranous angina in which the organisms were present. These early reports have been followed by a large number of corroborative observations which testify to the occurrence in preponderating numbers of fusiform bacilli and spirilla in certain cases of stomatitis and angina. A large number of these observations have appeared in French literature, many in the German and American, and a few in the English. This indicates the wide distribution of the organisms in question. The infrequency with which the disease has been recognized has probably been due to failure to make direct examinations of the exudate from pseudomembranous lesions of the mouth and throat. Because diphtheria bacilli are not detected with any certainty by such examinations, the custom of depending upon cultures quite exclusively has become almost universal, and as the fusiform bacilli and spirilla do not grow to any extent upon the medium usually employed for the detection of diphtheria bacilli, they have been largely overlooked.

The fusiform bacilli and spirilla have also been described in connection with hospital gangrene by Vincent and Matzenauer, and the bacilli alone by

* Received for publication, April 21, 1905.

Coyon. The two organisms have been found in association with noma by Matzenauer, Seiffert, Perthes, Rosenberger, and others. The bacilli alone have been noted by many observers in cases of noma. In several instances the organisms have been found in the pus from fetid abscesses about the mouth (Veszprémi, Silberschmidt, Seitz, Lichtwitz, and Sabrazes). Rodella found the fusiform bacilli in the fetid contents of a subpectoral abscess. Silberschmidt observed the two organisms in pus from a necrotic phlegmon of the thigh and from a bronchiectatic cavity, and the bacilli alone in a brain abscess. Veillon and Zuber demonstrated the bacillus by means of pure cultures in two cases of appendicitis, and designated it "bacillus fusiformis." Niclot and Marotte found the bacilli and spirilla in the intestinal contents in a case of dysentery in a dog, and Angelici says that the bacillus is present in various morbid processes throughout the alimentary canal in man and the lower animals. Rona found the two organisms in 15 cases of gangrenous ulcers of the penis.

There appears to be no case reported in which the fusiform bacillus either alone or in association with the spirillum, has been found in connection with a morbid condition in man without the simultaneous presence of other bacteria. Cocci are almost constantly present. In the ulceromembranous anginas, the two bacteria under consideration have been observed in connection with diphtheria bacilli by Abel, Baron, Bernheim, Vincent, Niclot and Marotte, Auchè, Simonin, de Stoecklin, Gallois and Courcoux, and Oberwinter. Abundant fusiform bacilli, but no spirilla, were found together with diphtheria bacilli in one case by Beitzke and in six cases by Oberwinter. The latter author also observed three cases of diphtheria in which smears showed almost only fusiform bacilli and spirilla. That the organisms occur in syphilitic lesions of the mouth and tonsil is shown by the observations of Baron, Vincent, Freyche, Graupner, Hallopeau and Apert, Salomon, and Wolf. Hallopeau and Apert believe that these organisms may locate upon wounds, as after tonsillotomy, or upon the gums of scorbutics, and may complicate mercurial stomatitis.

Abel was successful in demonstrating both varieties of bacteria in healthy mouths, especially between the gums and teeth. Gross found bacilli and spirilla in small numbers upon the normal tonsils of 11 of 13 persons. It is generally stated that the spirilla described in these cases are the "spirochaeta denticola" of Miller. Corresponding spirilla were found by Rona in small numbers on healthy genitalia, and Rosenberger found spirilla closely resembling those in ulcerative angina, but without the associated bacilli, in the vaginal secretion of children and adults.

In most of the cases, the bacilli and spirilla have been recognized in smear preparations made from the seat of disease, and in stained microscopic sections of tissues. Usually efforts to cultivate the organisms have resulted in failure. A number of investigators have grown the bacilli in mixed cultures: Angelici in media containing acetic acid, Gross in ascites serum, Niclot and Marotte in media containing sera, Seitz in ordinary broth and in the water of condensation in serum-agar tubes, Silberschmidt in broth and ascites-broth, Veszprémi in broth mixed with various sera, Seiffert, Perthes, and Brüning in deep agar tubes. Some of these authors also obtained a

simultaneous growth of spirilla: Niclot and Marotte in pleural fluid and broth, and other mixtures of sera, Silberschmidt in broth containing one per cent of acetic acid, Veszprémi in media containing rabbit serum, Seiffert and Perthes in agar, Netter in ascites fluid and defibrinated pleural fluid. There does not appear to have been any successful pure cultivation of the spirilla. Pure cultures of fusiform bacilli have been obtained by Veillon and Zuber, and by Ellermann.

The present description is based upon the study of the organisms from the following sources: Three cases of ulceromembranous stomatitis; five cases of ulceromembranous angina; two cases of combined angina and stomatitis; one case of noma of the cheek; one case of diphtheria; saliva and tartar from 18 healthy mouths. Three of these cases, one of angina and one of stomatitis, and the one of diphtheria, occurred in the department of contagious diseases of the Cook County Hospital in the service of Dr. Wm. L. Baum. Most of the other cases were observed in the hospital of the Memorial Institute for Infectious Diseases, in the service of Dr. Frank Billings and Dr. Alexander F. Stevenson. To these gentlemen we wish to express our thanks for the opportunity to study the cases. Two cases were observed in private practice.

The clinical side of the subject will be reserved for a separate report, and the case of noma will be dealt with in detail at a future time.

EXAMINATION OF MATERIAL FROM THE LOCAL LESIONS.

In smear preparations made from the seat of disease, bacilli and spirilla were found which corresponded with those described by other authors.

The bacilli are long, slender rods with pointed ends, somewhat larger in the middle. Sometimes the ends are rounded and the rod is rather thick. The rods are sometimes slightly bent, and occasionally take the form of the letter S. The length is usually from 6 to 12 μ, but sometimes filiform elements of considerable length are encountered. The bacilli are usually scattered quite uniformly throughout the preparation, and often occur as pairs end-to-end, sometimes forming more or less obtuse angles. At times they are seen in irregular clumps, or arranged radially about a common central point, or in rows somewhat similar to

diphtheria bacilli. They stain fairly well with Loeffler's solution of methylene blue and anilin-water solution of gentian violet, but best with carbolfuchsin. With the less intense stains. especially in the larger forms, there are often portions of variable size and shape which stain faintly. These have been described by many observers, and sometimes spoken of as vacuoles. They have also been mistaken for spores, but they do not stain like spores. Vincent has described giant forms of the bacilli. With Lugol's solution of iodine there is no staining, nor starch granules. As stated by Gross, the bacilli contain no Ernst granules. No motility could be detected. This corresponds to the statements of most authors (Vincent, de Stoecklin, Niclot and Marotte, and others). Some observers, however, describe the bacilli as motile (Baron, Abel, Bernheim, Graupner, Hess, Sobel and Herrman). Graupner has illustrated the bacilli with stained peritrichous flagella. He found that motility was rapidly lost, even under favorable conditions in 20 minutes. Beitzke explains the varying expressions regarding motility by this fact, but it is better accounted for by Ellermann, who cultivated the shorter, curved forms in pure culture, and found them to be motile spirilla.

The bacilli do not stain by Gram's method. Several authors say that rather prolonged action of alcohol is required to accomplish complete decolorization. Vincent, Abel and Niclot and Marotte say that it is stained by Gram's method, but this is contradicted by all other authors.

The number of bacilli is variable. In the earlier stages of ulceromembranous angina and stomatitis, they are most abundant, and decrease as the process of recovery advances. When the bacilli and spirilla are most abundant, other forms of bacteria are present in small numbers. As the two organisms decrease, the associated bacteria usually increase. In normal mouths the fusiform bacilli are present in small number in smears from the saliva, tongue and gums. In the case of diphtheria they were to be found only after considerable search.

The spirilla, also spoken of as spirochaetae, which are associated with the fusiform bacilli in a large proportion of instances, are long, delicate, and present three to six or eight turns. They

stain uniformly, and much less intensely than the bacilli, and in faintly-stained preparations might be overlooked. They do not stain by Gram's method being much more quickly decolorized, than the bacilli. They are usually quite actively motile, but sometimes not. Vincent says that some are immobile or only slightly motile, while others are very actively motile. Niclot and Marotte state that they rapidly lose their motility when exposed to cold. The association of spirilla with the bacilli was observed in all the cases studied. In general their number corresponded to the number of bacilli. In the mouths of many healthy persons, what appeared as the same spirilla were found, especially about the gums, often in enormous numbers.

Most authors believe the fusiform bacilli and spirilla to be entirely distinct varieties of bacteria and that they act in symbiosis, the spirilla serving to enhance the virulence of the bacilli. Vincent, Niclot and Marotte, Baron, Hess, Oberwinter, etc., have observed that those cases of ulceromembranous angina in which only the bacilli are found are milder than those in which the two organisms are associated. In cases in which deeper destruction of tissues occur, the spirilla are said to be constantly present. In the case of noma from which material for study was obtained, fusiform bacilli and spirilla were present in the nasal discharge from the beginning, and later in the ulcerative lesions of the gums and cheek.

Some observers have maintained that the bacilli and spirilla are different forms of one organism (Seiffert, Perthes, Sobel and Herrman, and Krahn). After careful examination of many preparations, both direct from man and from pure and mixed cultures, the writers have been unable to find any evidence to support these assertions. Most authors consider the bacilli and spirilla distinct organisms.

MIXED CULTURES OF FUSIFORM BACILLI.

In some of our earlier aërobic cultures it was observed that there was a slight growth of bacilli in the fluid of condensation at the bottom of tubes of Loeffler's blood serum mixture, and in tubes of ascites fluid. It was soon found that they grew best in sugar-free broth, either alone or combined with horse serum or ascites

fluid. Angelici says that sugar inhibits the growth of the bacilli in mixed culture, and Niclot and Marotte found that they did not grow in sugar broth. After dextrose-free broth was employed, there was no failure in obtaining the bacilli in association with cocci, and almost always streptococci. In aërobic cultures in dextrose-free broth made direct from materials containing the bacilli there was little increase of the bacilli during the first 24 hours. During the second 24 hours there was a great increase, even when the inoculated material contained very few bacilli, as in saliva from healthy mouths, and from the case of diptheria. The cultures then emitted a foul odor. Coverslip preparations showed fusiform bacilli together with other bacilli and especially with cocci. The bacilli presented about the same appearances as they did in smears made direct from the patient. The reaction of the culture fluid after 48 hours was alkaline to litmus. In a mixture of ascites fluid and dextrose-free broth (one to three) the growth was similar to that just described but the fetid odor was more pronounced. Most authors mention the foul odor of such cultures. In one lot of ordinary nutrient peptone broth, there was abundant growth of the bacilli in mixed cultures, while in another lot there was no apparent growth. The growth in the water of condensation at the bottom of tubes of Loeffler's blood serum mixture was variable, sometimes succeeding in a limited degree, and again failing.

In dextrose-free broth the fusiform bacilli grew in mixed cultures with no exclusion of oxygen. In this respect the fusiform bacilli resemble many other anaërobes which grow readily in association with suitable aërobes in fluid media without exclusion of oxygen. In the mixed cultures described, the fusiform bacilli rapidly die, after a week or 10 days scarcely any can be detected. The fusiform bacilli did not increase appreciably upon solid media in mixed cultures with or without the exclusion of oxygen.

The fusiform bacillus was grown in cultures in association with other bacteria from the following sources: Two cases of ulceromembranous stomatitis; four cases of ulceromembranous angina; one case of combined angina and stomatitis; one case of noma; one case of diphtheria and 13 normal mouths.

MIXED CULTURES OF SPIRILLA.

The spirilla were grown in combination with fusiform bacilli, streptococci and the other bacteria from a tonsillar concretion, and from tartar from six healthy mouths. The inoculated material contained many spirilla. The medium employed was human pleuritic exudate and broth in one case, and in the others broth which contained no fermentable sugar, but a small amount of muscle sugar. The increase of spirilla was always limited, and the exclusion of oxygen did not appear to influence the growth. In one instance a subculture was successful. The spirilla were not grown on solid media, and never in pure culture.

PURE CULTURES OF FUSIFORM BACILLI.

Pure cultures of fusiform bacilli were obtained from three cases as follows: One case of ulceromembranous angina; one case of ulceromembranous stomatitis; one case of diphtheria of the tonsil. In the cases of stomatitis and angina the fusiform bacilli and spirilla were very numerous in smear preparations from the exudate; both were typical cases clinically. In the case of diphtheria the bacilli were present in small numbers in the smears. Typical diphtheria bacilli were cultured from this case in abundance, and there was a prompt improvement after the administration of diphtheria antitoxin. In the two former cases, material from the local disease was smeared over the surface of a series of slants of horse-serum-agar. After anaërobic growth at 37° for three to five days, the colonies of fusiform bacilli appeared as very delicate, whitish disks one to two mm. in diameter, resembling colonies of streptococci. By inoculation from such colonies pure cultures were obtained. In the case of diphtheria a culture was first made into sugar-free broth and ascites fluid (three to one), and when the bacilli had become abundant, smear cultures were prepared upon slants of serum-agar, as in the other case. Unsuccessful efforts were made to obtain pure cultures from a few other cases. In a 48-hour broth culture containing a great number of the bacilli, a good many are probably already dead, as very few colonies develop when the broth culture is smeared over serum-

agar slants. In many instances colonies were found to contain the bacilli and streptococci in association.

The three pure cultures possessed the following characteristics:

CULTURAL AND BIOLOGICAL PROPERTIES.

They were obligate anaërobes, and grew at 36°, but not at all at room temperatures. They were non-motile.

All the cultures in the following description were grown by Wright's method, by saturating the cotton stopper with a strong solution of pyrogallic acid in a five per cent solution of sodium hydroxid, and closing the tube with a tightly fitting cork, sealed with paraffin.

Slant of horse-serum agar. After 24 or 48 hours a delicate, whitish, confluent growth appears over the inoculated surface. In the water of condensation the flocculent growth collects at the bottom, leaving the fluid clear.

Slant of ascites-agar. At the end of 24 to 48 hours there appears a delicate continuous, white growth, frequently with delicate colonies, one and two mm. in diameter along the edge of the streak. A flocculent growth collects at the bottom of the fluid of condensation.

Loeffler's blood serum. In 24 hours a barely perceptible growth appears along the line of inoculation, which increases for three or four days. At its maximum growth it is slightly moist, a little irregular, or granular on the surface, continuous, resembling much some streptococcus cultures.

Agar slant. In the first 24 hours there is no visible growth. In 48 hours there forms a very delicate, whitish growth, limited to the line of inoculation, and appearing as a cloud upon the surface.

Glycerin agar slant. After 24 or 48 hours there appears a very delicate, whitish, cloudy growth upon the surface, following the line of inoculation, with pin-point sized colonies at the border. The growth here is less abundant than upon plain agar, and the agar looks as if it had become opaque where the growth is located. One of the cultures failed to grow upon this medium.

Stab in glucose agar and ascites fluid (three to one), with a layer of water agar above. Beginning in 24 hours, and increasing for three days, an abundant, grayish-white, opaque growth develops all along the needle track. There is no gas formed.

Glucose agar stab (with overlying layer of water agar). The growth is similar to the preceding but less profuse. There is no growth unless considerable culture is inoculated.

Litmus milk. No appreciable growth of bacilli occurs.

Litmus milk and ascites fluid. After a week there is a growth of bacilli, the medium being decolorized in two days. When oxygen was admitted, the medium gradually assumed a red color.

Potato smeared with ascites fluid. No growth.

Egg. An unbroken egg was inoculated through a small opening with proper precautions to prevent contamination, and the opening sealed. After seven days the white of the egg was turbid, the yolk fluent, and there were present abundant fine bacilli and filaments. The latter were sometimes stained irregularly in segments with carbolfuchsin, causing an appearance suggestive of streptococci. There was no foul odor.

Dextrose-free broth. A slight flocculent growth appears after 24 hours. This increases for three or four days, and settling to the bottom, leaves a clear fluid above. On agitation the growth rises as a rope at first, but is readily distributed throughout the fluid without visible granules.

Dextrose-free broth and ascites fluid (three to one). The growth is similar to the preceding but more abundant. The upper fluid retains a slight smoky opacity or opalescence.

Dextrose-free broth and horse serum (three to one). The growth is similar to the former but the upper fluid is apt to be perfectly clear.

Plain nutrient broth. There is no growth. Acetic acid added to broth seemed to interfere with rather than to aid the growth of the bacilli. All the cultures upon media containing blood serum and ascites fluid gave off a very offensive odor. Filter-paper moistened with a dilute solution of lead acetate is turned brown in the upper part of such culture tubes, indicating the production of sulphides.

If varying amounts of glucose are added to tubes of sugar-free broth, it is found that the bacilli do not grow when the percentage of glucose is 0.5 per cent or higher. If the proportion is 0.25 per cent or less, growth occurs. If, however, ascites fluid is mixed with the broth, growth occurs when two per cent of glucose is present.

Two of the cultures were inoculated into plain broth without and with the addition of one and two per cent glucose, dextrose, levulose, and lactose, and two and five per cent glycerin. In none was there any growth after 24 hours. Corresponding tubes with one-third the bulk of ascites fluid added showed abundant growth after the same length of time. After a longer time in levulose broth there is usually a limited growth.

The results of the aërobic growth of the fusiform bacillus alone and in association with two other bacteria in sugar-free and one per cent dextrose broth is shown in the accompanying table.

Cultures			Media	After 48 Hours Growth	
Bac. fusiformis	Streptococcus from angina	Pseudodiph. bac. fr. case of noma		Reaction to Litmus	Cover-slip preparations
+	−	−	Sugar-free broth	Faintly alkaline	Very few fusiform bacilli
+	−	−	1% dextrose broth	Faintly alkaline	No growth
+	+	−	Sugar-free broth	Strongly alkaline	Moderate increase of bac. Much increase of strep.
+	+	−	1% dextrose broth	Strongly acid	No bacilli Great increase of strep.
+	−	+	Sugar-free broth	Strongly alkaline	Great increase of fusiform bac. Few pseudo-d. bac.
+	−	+	1% dextrose broth	Strongly acid	Slight or no growth of fusiform bac. Great increase of pseudodiphtheria bac.

These results seem to explain why the fusiform bacilli sometimes fail to grow in mixed cultures when the nutrient fluid contains sugar, the rapid production of acid by streptococci and other bacteria preventing growth of the bacilli. The outcome in such cases would depend upon the varieties of bacteria simultaneously inoculated. Varying amounts of sugar in ordinary nutrient broth may account for varying degrees of resulting acidity, and so for growth or failure of growth of fusiform bacilli in such media when inoculated with material containing various bacteria together with the bacilli.

The viability of the cultures was not reduced after repeated transplantations for three months.

MORPHOLOGY AND STAINING PROPERTIES.

In dextrose-free broth there are delicate pointed rods, staining uniformly, usually straight, sometimes bent. They are like those in smears from the local diseases, but not so large in the center, being of nearly the same diameter throughout. Similar bacilli occur in cultures upon plain nutrient agar and ascites agar. In ascites-broth and horse-serum broth the bacilli are slender, with rounded ends and center not enlarged, varying much in length from short forms to longer filaments; often in pairs, end-to-end or in longer chains, usually straight, sometimes bent and wavy. They stain evenly and intensely with carbolfuchsin. When they have grown upon slants of ascites-agar, horse-serum-agar and Loeffler's blood serum mixture they appear as long delicate filaments. With carbolfuchsin they stain rather faintly, but within the rods at irregular intervals are seen deeply stained round or oval bodies. Some filaments are larger than the average, and sometimes the appearance suggests the lateral fusing of two or three filaments, giving rise to giant forms. Sometimes branching was suggested, but none could be with certainty detected. In Loeffler's serum cultures there were sometimes long, filamentous forms which exhibited alternate deep and faintly stained segments, looking like a string of short bacilli. In cultures upon all solid media, there occur individuals which stain faintly, containing the more deeply stained round bodies. Cultures upon ascites-agar sometimes show only evenly stained, short and medium long rods with pointed ends, like those occurring in sugar-free broth. Methylene blue stains the rods fairly well, and after staining with Loeffler's solution, the more deeply stained bodies appear as when carbolfuchsin is employed.

They did not stain by Gram's method, but unless thoroughly decolorized with alcohol they retained a very faint, bluish color.

The bacteria which have been cultivated in pure culture and described as fusiform bacilli, may be discussed somewhat in detail.

Veillon and Zuber in 1898 described the "bacillus fusiformis," which they obtained from two cases of appendicitis. It was a non-motile, obligate anaërobe, which did not form spores, and grew at room and body temperature. In pus it occurred as large, spindle-shaped rods, often in pairs. In cultures it was usually similar in form, but also presented elongated, swollen and granular forms. It stained poorly with anilin dyes, and not at all by Gram's method. It grew rapidly in sugar-agar; after 24 hours small whitish colonies appeared, which later became gray and brownish. The colonies were lenticular, opaque and sometimes became quite large. There was limited formation of foul gas. Gelatin was not liquified. Broth rapidly became markedly cloudy with a dense sediment. In guinea pigs and rabbits it caused small abscesses which healed.

Ellermann in 1904 reported the first successful cultivation of a fusiform bacillus from cases of ulcerous angina and stomatitis. It was a non-motile, obligate anaërobe. In cultures it appeared as slender, straight rods, with pointed ends, often in pairs, usually 5 to 12 μ long, and occasionally as very long filaments. Swelling at the center was not usual. The bacilli stained poorly and unevenly, and were not stained by Gram's or Weigert's methods. They contained no Babes-Ernst granules.

The nutrient medium employed was serum-agar (two parts of agar and one part fluid horse-serum). Colonies appeared after two days, reaching a size of 1 to 1.5 mm. The smaller had a felted appearance; larger ones were circular, and the largest were often prismatic and of a pale yellowish color. The nutrient medium became cloudy. The cultures possessed an offensive odor, but gas bubbles rarely formed. In serum-broth after 24 hours, large, white flocculi formed which later sank to the bottom. Upon the surface of serum-agar there developed small colonies

resembling those of streptococci, or a finely granular, continuous growth. There was no growth upon ordinary agar or broth. The viability was not reduced after nine transplantations.

These two reports are the only ones found which deal with pure cultures of bacilli which resemble the ones described in this paper, Each description is at variance in certain particulars from the other and from the one herein presented, and it is difficult to decide whether the same organism has been studied in each instance or not. In the absence of accurate information as to the variability of certain physiologic properties in these bacteria it is not possible to decide whether certain differences are permanent and essential. In agar cultures, the amount of bacteria inoculated is important.

It appears that at least the organisms studied by Ellermann are the same as those here described.

INOCULATION EXPERIMENTS.

Pure cultures of the bacilli were injected subcutaneously and into the muscles in rabbits and guinea pigs without any result. Veillon and Zuber, with pure cultures of the "bacillus fusiformis" produced small abscesses in rabbits and guinea pigs, which healed. Ellermann does not appear to have tested the pathogenic properties of his cultures.

With the mixed cultures containing fusiform bacilli, we have produced abscesses in guinea pigs by intra-muscular injection. The pus contained the bacilli together with cocci and they were again cultivated from the pus in impure growth. A mixed culture from a case of extensive ulceromembraneous angina was injected subcutaneously in the ear of a small, sickly rabbit. There resulted an extending gangrene of the skin, terminating in the animal's death. After death no fusiform bacilli or spirilla could be found, but only diplo- and streptococci. Mixed cultures containing a growth of fusiform bacilli and spirilla were also injected into the muscles and subcutaneous tissues of guinea pigs with resulting formation of abscesses. In the contents of such abscesses bacilli and spirilla were present and were again cultivated together with cocci. If cultures contained no spirilla, but only fusiform bacilli with cocci, the results were similar.

RELATION OF FUSIFORM BACILLI AND SPIRILLA TO MORBID PROCESSES.

The injection into experimental animals of morbid materials containing fusiform bacilli, with or without spirilla, obtained from infected wounds, in hospital gangrene (Matzenauer, Vincent, Coyon), from necrotic tissues in noma (Guizzetti, Perthes), from ulceromembranous angina and stomatitis (Niclot and Marotte, Carnot and Fournier, Graupner), and from fetid abscesses (Silberschmidt and Veszprémi) have often been followed by localized necrosis and formation of abscesses. Similar results have been noticed to follow the injection of mixed cultures from the same sources. The materials and cultures which have been injected have always contained, not only fusiform bacilli with or without spirilla, but also various other bacteria among which usually have been included cocci, and often streptococci. When such materials are injected it is obviously impossible to determine which of the many forms is most largely responsible for the result. With our accurate information regarding the action of the pyogenic cocci, and their power to produce necrosis and suppuration, it would be reasonable to assume that they are responsible for the results, at least in part.

The strongest evidence in favor of a causal relationship between the fusiform bacilli and the necrotic processes with which they are associated has been furnished by the microscopic examination of the sections of the involved tissues. In tissues from noma cases, filamentous organisms have been demonstrated by many observers at the line of advancing necrosis. Here they are found in great numbers, and a few are also seen in the tissues which are still not visibly altered. Gross and Krebs have also made similar observations in tissues from ulceromembranous angina. The filamentous organisms described by many authors, and studied by us in nomatous tissues bear a close resemblance to the form assumed by the fusiform bacilli in certain artificial pure cultures, as described in this report. The identity of these bacteria in the tissues with the fusiform bacillus cannot be said to be indisputably established.

It is not unlikely that future study will show that what have been spoken of as "fusiform bacilli" are not a single variety of

PLATE 16.

FIG. 1.

FIG. 2.

FIG. 3.

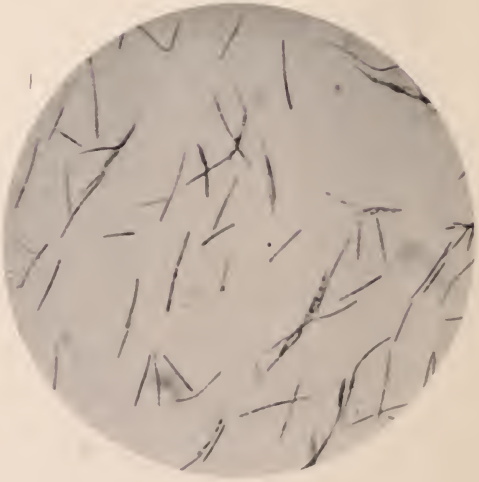

FIG. 4.

bacterium, but represent several distinct varieties as suggested by Beitzke. Now that the common statement that these organisms cannot be grown in pure culture has been shown to be false, it may be hoped that further study with pure cultures will shed light upon the relationship of the bacteria to the diseases with which they are associated.

Note.—Since the above was completed and presented before the meeting of the American Association of Pathologists and Bacteriologists, April 21, 1905, there has been received a further contribution by Ellermann (*Centralbl. f. Bakt.* Abt. 1, Originale, 1905, 38, p. 383.) to whose preliminary report reference has already been made. In the tissues of the uvula from a case of ulceromembranous angina, and in those from a case of gangrenous stomatitis, he found fusiform bacilli in the zone separating necrotic and living tissues. In the latter case spirilla in abundance were also demonstrated in this location. These observations are in agreement with those of previous observers in noma and of Gross and Krebs in ulcerative angina. Ellermann has also produced small abscesses in rabbits with pure cultures of the bacillus.

BIBLIOGRAPHY.

In connection with his excellent résumé of the literature bearing upon the fusiform bacillus, Beitzke (*Centralbal. f. Bakt.*, Abt. I., Referate, 1904, 35, p. 1) has collected references to 113 publications. We have given only those to which we have referred and which are not found in his list.

ANGELIÇI. *Il Policlinico* (Rome), Feb. 20, 1904, quoted by Rosenberger.

AUCHÉ. *Gaz. hebd. d. sc. méd. de Bordeaux*, Nov. 1903, cited by Dubar. *Le Progrès Med.*, 1904, 20, p. 17.

BRÜNING. *Jahrbuch f. Kinderheilk.*, 1904, 60, p. 631.

ELLERMANN. *Centralbl. f. Bakt.*, Abt. I., Originale, 1904, 37, p. 729.

GUIZZETTI. *Il Policlinico*, 1896, 405; Abstract, *Baumgarten's Jahresb.*, 1896, 2, 495.

MATZENAUER. *Archiv f. Dermat. und Syph.*, 1902, 60, p. 373.

NETTER. Cited by Raoult and Thiry. *Revue de Laryng.*, 1898, No. 30, p. 881.

ROSENBERGER. *Amer. Med.*, July 23, 1904, p. 161.

VESZPRÉMI. *Centralbl. f. Bakt.*, Abt. I., Originale, 1905, 38, p. 136.

EXPLANATION OF PLATE 16.

(Photomicrographs of smears stained with carbolfuchsin; × 1200.)

FIG. 1. Pure culture of fusiform bacillus grown five days anaërobically in dextrose-free broth.

FIG. 2. Pure culture of fusiform bacillus grown three days anaërobically on ascites-agar slant.

FIG. 3. Pure culture of fusiform bacillus grown three days anaërobically on Loeffler's blood-serum.

FIG. 4. Pure culture of fusiform bacillus grown three days anaërobically on ascites-agar slant.

A QUANTITATIVE STUDY OF HEMOLYTIC SERUM.*

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ONE of the most important problems of experimental medicine today is the determination of the rôle of physico-chemical laws in the chemistry of disease. The controversy over this question between Ehrlich on the one side and Arrhenius and Madsen on the other, touches many problems of practical medicine. Unfortunately, most of the toxic and antitoxic substances with which these investigators are working, are substances not yet isolated in a state of purity, are of unknown composition and probably of great complexity. Hence their study offers many difficulties.

It was thought that if a "border line" phenomenon were studied, some problem lying partly in the well-trodden field of inorganic chemistry, where the application of physico-chemical laws cannot be gainsaid, light might be thrown on the more complex phenomena now engaging the attention of these workers. For this reason, and at the suggestion of Dr. Hektoen, the study of the neutralization of a hemolytic serum with certain salt solutions was undertaken.

It was found comparatively easy to modify the technique of the hemolytic experiment so as to give to it an accuracy equal to that of the most exact hemoglobin determination, and by this means to plot neutralization curves for such sera. These curves, however, showed unlooked-for complexities.

In order to interpret them it was found necessary to plot corresponding curves showing the changes in hemolytic power as each of the prominent serum components varies. These preliminary curves are of such interest that it is thought best to embody them in a separate report. The present paper deals only with these curves.

*Presented before the American Association of Pathologists and Bacteriologists, April 22, 1905. Received for publication May 5, 1905.

NOMENCLATURE.

So much confusion has resulted from failure to separate demonstrated facts from hypotheses and theories in immunity, that it is thought best to define certain terms used in this paper. The term **amboceptor** is used to designate the specific immune body present in an immune serum. This is the "substance sensibilatrice" of Bordet, the intermediary body of Ehrlich, the "copulâ" of Müller. It is a thermostable substance, resisting heat to at least 57° C. for over an hour. By itself it has no bactericidal or hemolytic action. It produces, however, certain physical or chemical changes in bacteria and other cells, which render them susceptible to the action of a second serum component, the complement. Cells thus rendered susceptible are spoken of as being **sensitized**.

The **complement** is a normal component of serum. It is the "alexin" of Bordet, the "cytase" of Metchnikoff. It is a thermolabile substance, usually destroyed by heat to 55°C. for 30 minutes. By itself it has no bactericidal or hemolytic action. It is capable of producing such action only in the presence of amboceptor or when acting on cells previously sensitized by amboceptor.

These terms are selected because they are the ones most commonly used in American medical literature. They are used here simply as names for unknown chemical substances, with no implication as to the method of action or interaction of these substances.

MATERIAL AND TECHNIQUE.

The serum used in this study was obtained by immunizing goats by repeated intraperitoneal injections of washed sheep corpuscles. This serum was selected because Hektoen and Ruediger found that normal goat serum is without hemolytic action on sheep corpuscles, and because Ehrlich and Morgenroth[1] report that after immunization such serum has little or no agglutinating action, an action that interferes seriously with exact hemolytic work.*

[1] *Berl. klin. Wchnschr.*, 1899, 36, p. 481.

*Of the five normal goats used, four showed no hemolytic action on sheep corpuscles. The fifth gave a slight trace of hemolysis when its serum was used in large amounts. Of the two immune goats, one gave practically no agglutination of these corpuscles. The second gave at first a moderate amount of agglutination, which amount became less and nearly disappeared as the serum increased in hemolytic power.

Blood was drawn from the jugular vein of these animals by means of a sterile hypodermic needle through sterile rubber tubing, into a sterile Erlenmeyer flask, a suction-pump being used to hasten the flow. The sheep's blood was at once defibrinated by gently stirring it with a sterile glass rod or by shaking it with sterile glass beads. The goat's blood was allowed to clot, and was kept in a refrigerator till the serum separated. Serum free from complement was prepared by heating this serum to 57°C. for one hour.

The salt solution used in the work was made from water redistilled from glass, and extra pure NaCl obtained from the Department of Chemistry. The solution was eighth-molecular in strength and was sterilized in the autoclave* before being used.

In performing the hemolytic experiment, the following technique was finally adopted and was used in all the work herein reported. Test-tubes of a uniform internal diameter of 1.3 cms. were selected, were carefully cleaned, plugged with absorbent cotton made non-absorbent by treatment with a weak ethereal solution of paraffin, and sterilized by dry heat (150° C. for one hour). With strictest aseptic precautions the sera and other solutions to be used were measured out into these test-tubes, and the volumes in the various tubes made up to a uniform amount, five c.c. The tubes were repeatedly shaken to insure a thorough mixing of the solutions.

After standing for an hour at room temperature, 3.5 c.c. of a seven per cent suspension of washed sheep corpuscles† were added to each tube and the tube immediately inverted once or twice to bring about a prompt, thorough and uniform mixing of the corpuscles and serum, the paraffin preventing adhesion of the fluid to the cotton plugs. The tubes were then incubated at 37.5° C. for three hours, after which they were placed in the refrigerator till the corpuscles settled. This generally took place in about 36 hours.

To estimate the amount of hemolysis a color-scale was prepared by laking corpuscles in distilled water in such amounts that 100 per

*Contamination by condensation water prevented by small beaker inverted over neck of flask.

†Corpuscles were washed four times in from five to six times their volume of salt solution. Supernatant liquid removed each time with a sterile capillary tube and suction pump. Calculated residual serum less than 0.00001 c.c. per hemolytic tube.

cent on the scale represented a complete solution of all corpuscles entering into a hemolytic tube. The fluids of this color-scale were placed in test-tubes the same diameter as the hemolytic tubes, and comparisons made in a darkened room by holding tubes side by side against a strongly illuminated piece of white paper as a background.

Before estimating the amount of hemolysis the liquid in each tube was mixed to insure a uniformity of color throughout. To do this the tube was gently tipped so as to allow a bubble of air to rise to the bottom. By careful manipulation, a thorough mixing can be brought about by this means, without disturbing the corpuscles at the bottom of the tube. After the first reading, the tubes were replaced in the refrigerator and the tints estimated again, 24 hours later. The average of the 36- and 60-hour readings was taken as the **observed hemolysis.**

To obtain the **true** or **calculated hemolysis** there was deducted from the observed hemolysis a percentage representing the sum of the tint of the serum originally added to the tube and the amount of autolysis taking place in control tubes. This sum was rarely over two per cent.

In the earlier experiments, considerable trouble was experienced on account of an excess of this autolytic action.* Part of this, it was thought, might possibly be due to faulty cleaning of glassware. To remedy this, every piece of glassware entering into the experiments was subjected to the following routine treatment. Before being used the first time, it was boiled for an hour in a strong solution of NaOH and soap, was scrubbed free from visible dirt, rinsed repeatedly in hot water and soaked for 24 hours in 10 per cent HCl. It was then rinsed in distilled water, soaked in distilled water for 24 hours, dried, plugged with chemically pure, non-absorbent cotton, and sterilized in the hot air sterilizer. During subsequent cleanings the same routine was used, except that the preliminary boiling in NaOH was usually omitted.

*The corpuscles of different sheep differ in the amount of this autolysis. The autolysis was so excessive with two sheep that the use of their corpuscles had to be discontinued. The autolysis apparently varies in the same animal with change in exercise and diet. The autolysis is completely inhibited by adding either of the antilytic salts $CaCl_2$ or $BaCl_2$. Other salts were not tried. One of the discarded sheep gave practically no autolysis when 0.85 per cent NaCl-solution was used. This was not true with the second discarded sheep. With all sheep, the spontaneous laking (autolysis) in $\frac{m}{8}$ NaCl decreased with repeated bleedings, and eventually practically disappeared.

A short study of autolysis showed that the larger part of it took place during the first 24 hours. Thus, with one sheep, during the first 24 hours' suspension in $\frac{m}{8}$ NaCl, there was a spontaneous laking of 1.5 per cent, during the second 24 hours a laking of 0.7 per cent, and during the third but 0.5 per cent. In order to reduce the autolytic tint to a minimum, the corpuscles were, therefore, freed from serum the afternoon of their withdrawal and allowed to stand in salt solution over night, when the major part of the autolysis took place. They were washed free from the tinted fluid immediately before being used the next forenoon. By this means control tubes were obtained, the combined autolytic and serum tint of which was often less than one per cent.

THE COMPLEMENT.

The first experiment to determine the changes in hemolytic power as the complement varies, was done with sensitized corpuscles. These were prepared by exposing washed corpuscles to heated immune serum, for an hour, at room temperature. The corpuscles were then freed from serum by centrifugation and washed repeatedly in salt solution. Increasing quantities of 20 per cent normal serum (complement) were added to a series of test-tubes and the volumes made up to a constant quantity (five c.c.) with 20 per cent heated normal serum (no complement). This gave a series of tubes each containing the same amounts of inorganic salts, serum albumin, etc., but differing in their content of the thermolabile substances, among which is the complement. 3.5 c.c. of a seven per cent suspension of sensitized corpuscles were then added to each tube, the tubes incubated, placed in the refrigerator, and tints estimated as described above. The result is recorded in Table 1 and shown graphically in Fig. 1.

TABLE 1.

DATE FOR COMPLEMENT CURVE. (See Fig. 1.)

	COMPLEMENT (measured in c.c.'s of normal serum)											
	0.0	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0 c.c.
36-hr. reading.	4.0	6.0	7.0	15.0	35.0	65.0	76.0	85.0	87.0	95.0	98.0	100.0
60-hr. reading.	4.0	6.0	8.0	17.5	45.0	70.0	80.0	85.0	88.0	95.0	100.0	100.0
Average	4.0	6.0	7.5	16.25	40.0	67.5	78.0	85.0	86.5	95.0	99.0	100.0
Autolysis + serum tint..	4.0						4.0					4.0
Calculated....	**0.**	**2.0**	**3.5**	**12.25**	**36.0**	**63.5**	**74.0**	**81.0**	**82.5**	**91.0**	**95.0**	**(100)**

TABLE 2.

Data for Complement Curves. (See Fig. 6.)

		Complement (Measured in c.c.'s of 66⅔ Per Cent Normal Serum)																				
		0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.125	1.25	1.375	1.5	1.75	2.0	2.25	2.5	2.75	3.0
Serum tint		**1.5**	....	....	....	....	....	....	....	....	....	**1.75**	....	....	....	....	....	**2.0**	....	....	....	**2.25**
0.00 c.c. Amboceptor (control)	36-hr. reading	1.5	....	....	....	....	1.5	....	....	....	....	1.5	....	....	....	1.5	....	2.0	....	2.5	....	2.5
	60-hr. reading	2.5	....	....	....	....	2.5	....	....	....	....	2.5	....	....	....	2.5	....	3.0	....	3.5	....	4.5
	Average	**2.0**	....	....	....	....	**2.0**	....	....	....	....	**2.0**	....	....	....	**2.0**	....	**2.5**	....	**3.00**	....	**3.5**
0.01 c.c. Amboceptor	36-hr. reading	2.0	2.5	2.5	3.0	3.0	4.0	4.0	5.0	6.0	7.0	7.5	9.0	12.5	14.0	13.0	15.0	16.0	17.5	19.0	22.5	25.0
	60-hr. reading	2.5	3.0	3.5	5.0	5.0	5.0	6.0	7.0	7.5	8.0	10.0	11.0	13.0	14.0	15.0	16.0	18.0	20.0	22.5	25.0	27.5
	Average	2.25	2.75	3.0	4.0	4.0	4.5	5.0	6.0	6.75	7.5	8.75	10.0	12.75	14.0	14.0	15.5	17.0	18.75	20.75	23.75	26.25
	Calculated	**0.25**	**0.75**	**1.0**	**2.0**	**2.0**	**2.5**	**3.0**	**4.0**	**4.75**	**5.5**	**6.75**	**8.0**	**10.75**	**12.0**	**12.0**	**13.25**	**14.5**	**16.0**	**17.75**	**20.5**	**22.75**
0.02 c.c. Amboceptor	36-hr. reading	2.0	2.0	3.0	4.0	7.5	9.0	15.0	17.5	27.5	35.0	37.5	42.5	45.0	47.5	52.5	59.0	65.0	67.5	72.0	75.0	82.0
	60-hr. reading	2.5	3.0	4.0	6.0	7.0	10.0	15.0	17.5	27.5	37.5	42.5	47.5	52.5	54.0	56.0	57.5	60.0	65.0	70.0	75.0	80.0
	Average	2.25	2.5	3.5	5.0	7.25	9.5	15.0	17.5	27.5	36.25	40.0	45.0	48.75	50.75	54.25	58.25	62.5	66.25	71.0	75.0	81.0
	Calculated	**0.25**	**0.5**	**1.5**	**3.0**	**5.25**	**7.5**	**13.0**	**15.5**	**25.5**	**34.25**	**38.0**	**43.0**	**46.75**	**48.75**	**52.25**	**56.0**	**60.0**	**63.5**	**68.0**	**71.75**	**77.5**
0.03 c.c. Amboceptor	36-hr. reading	2.0	2.5	3.0	5.0	8.0	15.0	25.0	32.5	47.5	60.0	65.0	68.0	72.0	75.0	77.5	80.0	82.5	85.0	88.0	93.0	98.0
	60-hr. reading	2.5	3.0	5.0	6.0	9.0	16.0	26.0	35.0	47.5	57.5	65.0	70.0	72.0	73.0	75.0	77.5	80.0	82.5	90.0	95.0	100.0
	Average	2.25	2.75	4.0	5.5	8.5	15.5	25.5	33.75	47.5	58.75	65.0	69.0	72.0	74.0	76.25	78.75	81.25	83.75	89.0	94.0	99.0
	Calculated	**0.25**	**0.75**	**2.0**	**3.5**	**6.5**	**13.5**	**23.5**	**31.75**	**45.5**	**56.75**	**63.0**	**67.0**	**70.0**	**72.0**	**74.25**	**76.5**	**78.75**	**81.0**	**86.0**	**91.75**	**95.5**
0.04 c.c. Amboceptor	36-hr. reading	2.0	3.0	3.5	7.5	15.0	27.5	42.5	55.0	62.5	68.0	70.0	75.0	80.0	80.0	82.5	82.5	87.5	92.5	95.0	100.0	100.0
	60-hr. reading	2.5	4.0	5.5	8.0	12.5	26.0	45.0	57.5	65.0	70.0	74.0	79.0	85.0	85.0	85.0	87.5	90.0	95.0	97.5	100.0	100.0
	Average	2.25	3.5	4.5	7.75	13.75	26.75	43.75	56.25	63.75	69.0	72.0	77.0	82.5	82.5	83.75	85.0	88.75	93.75	96.25	100.0	100.0
	Calculated	**0.25**	**1.5**	**2.5**	**5.75**	**11.75**	**24.75**	**41.75**	**54.25**	**61.75**	**67.0**	**70.0**	**75.0**	**80.5**	**80.5**	**81.75**	**82.75**	**86.25**	**91.0**	**96.25**	**96.75**	**(100)**
0.05 c.c. Amboceptor	36-hr. reading	2.5	3.0	5.0	12.0	21.0	45.0	60.0	64.0	70.0	75.0	79.0	80.0	82.5	86.0	86.0	89.0	95.0	100.0	100.0	....	....
	60-hr. reading	2.5	5.0	6.0	11.0	22.5	42.5	65.0	70.0	75.0	77.5	81.0	85.0	87.5	94.0	95.0	97.5	100.0	100.0	100.0	....	....
	Average	2.5	4.0	5.5	11.5	21.75	43.75	62.25	67.0	72.25	76.25	80.0	82.5	85.0	90.0	90.5	93.25	97.5	100.0	100.0	....	....
	Calculated	**0.5**	**2.0**	**3.5**	**9.5**	**19.75**	**41.75**	**60.25**	**65.0**	**70.25**	**74.25**	**78.0**	**80.5**	**83.0**	**88.0**	**88.5**	**91.0**	**95.0**	**97.25**	**(100)**	....	....
0.06 c.c. Amboceptor	36-hr. reading	2.0	3.0	6.0	15.0	27.5	60.0	75.0	80.0	85.0	90.0	90.0	91.0	96.0	95.0	95.0	100	100	....	....	....	....
	60-hr. reading	3.0	6.0	6.5	14.0	27.5	60.0	75.0	82.5	85.0	87.5	90.0	93.0	95.0	95.0	97.0	99	100	....	....	....	....
	Average	2.5	4.5	6.25	14.5	27.5	60.0	75.0	81.25	85.0	88.75	90.0	92.0	95.5	95.0	96.0	99.5	100	....	....	....	....
	Calculated	**0.5**	**2.5**	**4.25**	**12.5**	**25.5**	**58.0**	**73.0**	**79.25**	**83.0**	**86.75**	**88.0**	**90.0**	**93.5**	**93.0**	**94.0**	**97.25**	**(100)**	....	....	....	....
0.08 c.c. Amboceptor	36-hr. reading	2.0	3.0	10.0	25.0	58.0	85.0	90.0	92.5	95.0	100.0	100.0	....	....	....	....	....	....	....	....	....	....
	60-hr. reading	3.0	6.0	10.0	26.0	60.0	85.0	87.5	95.0	95.0	97.5	100.0	....	....	....	....	....	....	....	....	....	....
	Average	2.5	4.5	10.0	25.5	59.0	85.0	88.75	93.75	95.0	98.75	100.0	....	....	....	....	....	....	....	....	....	....
	Calculated	**0.5**	**2.5**	**8.0**	**23.5**	**57.0**	**83.0**	**86.75**	**91.75**	**93.0**	**96.75**	**98**	....	....	....	....	....	....	....	....	....	....
0.12 c.c. Amboceptor	36-hr. reading	2.5	6.0	17.5	52.0	85.0	100.0	100.0	100.0	....	....	....	....	....	....	....	....	....	....	....	....	....
	60-hr. reading	3.0	4.0	17.5	50.0	85.0	100.0	100.0	100.0	....	....	....	....	....	....	....	....	....	....	....	....	....
	Average	2.75	5.0	17.5	51.0	85.0	100.0	100.0	100.0	....	....	....	....	....	....	....	....	....	....	....	....	....
	Calculated	**0.75**	**3.0**	**15.5**	**49.0**	**83.0**	**(100)**	**100+**	**100+**	....	....	....	....	....	....	....	....	....	....	....	....	....

A similar experiment with non-sensitized corpuscles gave the curve in Fig. 2. In this experiment increasing quantities of $33\frac{1}{3}$ per cent normal serum were added to a series of test tubes, the volumes made up to a constant amount (three c.c.) with a $33\frac{1}{3}$ per cent heated normal serum, and the same amount of amboceptor (two c.c. 30 per cent heated immune serum No. 1) added to each. After thoroughly mixing and allowing to stand for an hour at room temperature, 3.5 c.c. of a seven per cent suspension of corpuscles were added to each tube and the tubes treated as above. The curve obtained in this way differs so little from that with sensitized corpuscles, that the use of sensitized corpuscles was discontinued in this work.

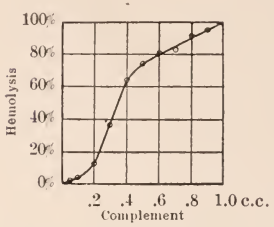
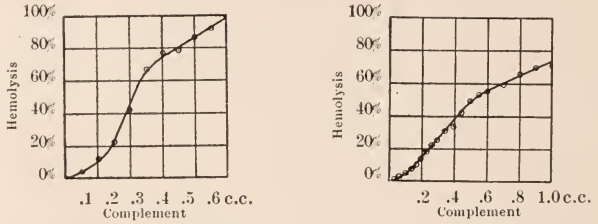


FIG. 1.—Complement curve with sensitized corpuscles.

A third curve, obtained in the same way but with a smaller constant amount of amboceptor (two c.c. 15 per cent heated immune serum No. 1) is shown in Fig. 3.



FIGS. 2 AND 3.—Complement curves with constant amboceptor.

To determine the influence of the amount of amboceptor on the nature of these curves, parallel curves were run, with the same varying amounts of complement but with different constant amounts of amboceptor. Two curves obtained in this way are shown in Fig. 4. In the upper curve 0.05 c.c. of heated immune serum (No. 2) was used as amboceptor; in the lower curve, but half that amount. A similar set of curves is shown in Fig. 5. The upper one was made with 0.15 c.c. of heated immune serum (No. 1), the lower with 0.075 c.c. of the same serum.

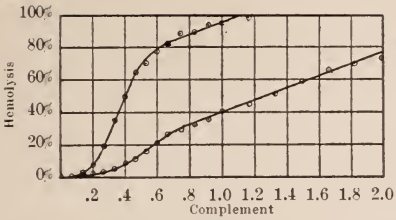


Fig. 4.—Parallel complement curves. (Immune Serum No. 2, Normal Serum No. 4.)

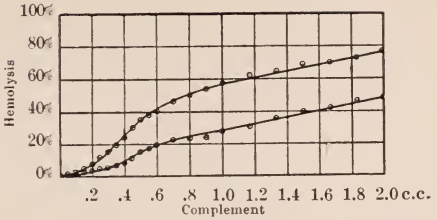


Fig. 5.—Parallel complement curves. (Immune Serum No. 1, Normal Serum No. 3.)

Two more extensive series of curves are shown in Figs. 6 and 7. The readings and calculations for the curves in Fig. 6 are given in Table 2.

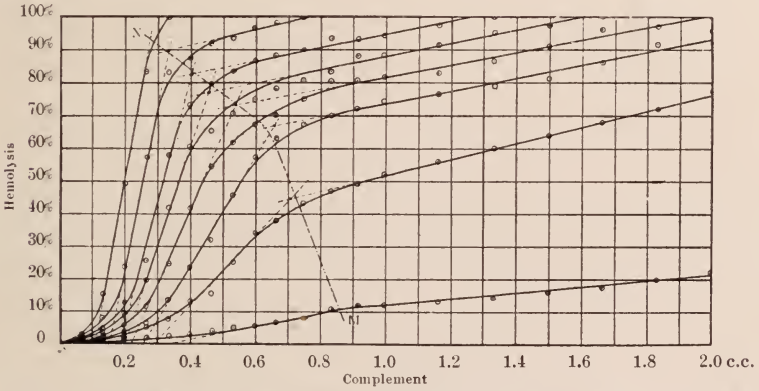


Fig. 6.—Parallel complement curves. (Immune Serum No. 2, Normal Serum No. 4.) The curves read from below upwards were made with the following amounts of amboceptor: .01 c.c., .02 c.c., .03 c.c., .04 c.c., .05 c.c., .06 c.c., .08 c.c., .12 c.c.

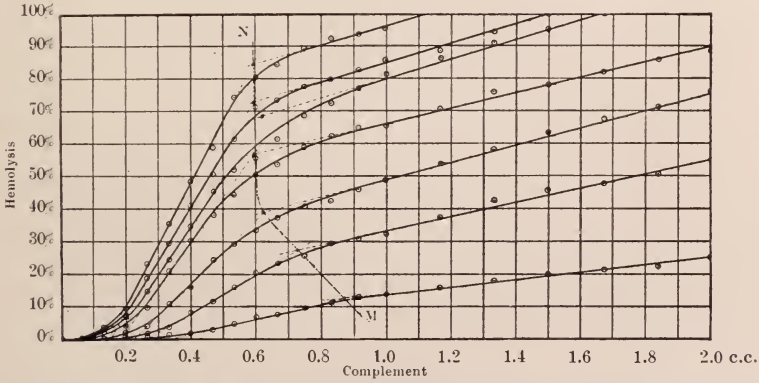


Fig. 7.—Parallel complement curves. (Immune Serum No. 1, Normal Serum No. 5.) The curves read from below upwards were made with the following amounts of amboceptor: .03 c.c., 0.6 c.c., .09 c.c., .12 c.c., .15 c.c., .18 c.c., .21 c.c.

From the curves thus far recorded, it is seen that the typical complement curve (Fig. 8) can be divided into three parts: an initial part AB, characterized by a slow, gradually increasing

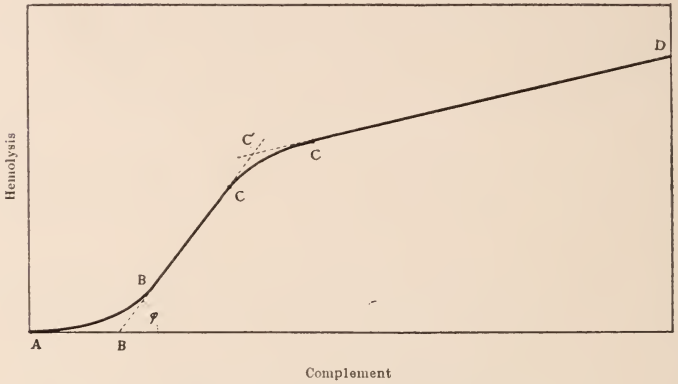


FIG. 8.—The typical complement curve.

rise; an intermediate part BC, characterized by a rapid, apparently uniform rise; and a third part CD, characterized by a slow, apparently uniform rise.

An examination of the curves in Figs. 6 and 7 shows that the length of the portion AB decreases with increase in the amount of amboceptor used, the relation between the two being expressed, at least approximately (using B′, the point most accurately determined experimentally) by the equation:

$$AB' = \frac{K^*}{1/\text{Amboceptor}} \qquad \text{(i)}$$

or,

$$AB' \times 1/\text{Amboceptor} = K \qquad \text{(ii)}$$

The values of K, calculated for the curves in Figs. 6 and 7, are given below, and show a striking uniformity in each series.

*A constant.

AB	Amboceptor	$\sqrt{\text{Amboceptor}}$	$K = AB \times \sqrt{\text{Amboceptor}}$
17.0	.01	.100	**1.7**
14.5	.02	.141	**2.0**
13.0	.03	.173	**2.3**
11.0	.04	.200	**2.2**
10.0	.05	.224	**2.2**
9.2	.06	.245	**2.3**
7.8	.08	.283	**2.2**
5.6	.12	.346	**1.9**
			Average, **2.1**
17.5	.03	.173	**3.0**
13.2	.06	.245	**3.2**
11.0	.09	.300	**3.3**
9.0	.12	.346	**3.1**
8.5	.15	.387	**3.2**
7.5	.18	.424	**3.2**
7.0	.21	.458	**3.2**
			Average, **3.2**

The rate of rise of the part BC increases with increase in the amount of amboceptor present. The relation between the two is expressed, at least approximately, by the equation:

$$\tan \phi = K \times \text{Amboceptor} \qquad \text{(iii)}$$

or,

$$\frac{\tan \phi}{\text{Amboceptor}} = K \qquad \text{(iv)}$$

The values of K, calculated for the curves in Figs. 6 and 7 are given below and are fairly constant in each series.

ϕ	$\tan \phi$	Amboceptor	$K = \frac{\tan \phi}{\text{Amboceptor}}$
12°	0.21	.01	(**21**)
48	1.1	.02	**55**
60	1.7	.03	**57**
66	2.2	.04	**55**
71	3.0	.05	**60**
73	3.3	.06	**55**
76	4.0	.08	**50**
80	5.7	.12	**48**
			Average, **54**

ϕ	$\tan\phi$	Amboceptor	$K = \frac{\tan\phi}{\text{Amboceptor}}$
14°	0.25	.03	**8**
31	0.6	.06	**10**
44	0.97	.09	**11**
55	1.4	.12	**12**
57	1.5	.15	**10**
60	1.7	.18	**9.5**
61	1.8	.21	**9**
		Average,	**10**

The height to which C rises increases with increase in the amount of amboceptor present. That this increase follows definite laws is shown in Figs. 6 and 7 by the curve MN, the locus of C′. The loci differ in the two series, indicating that the law

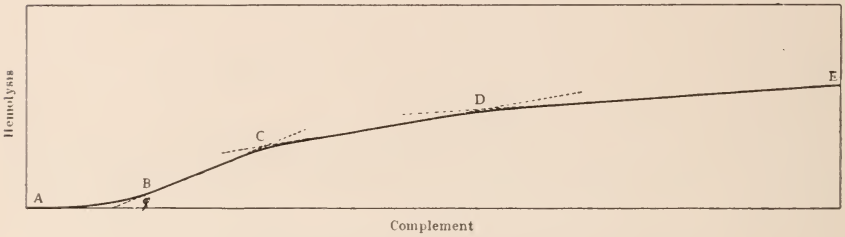


FIG. 9.—Complement curve with minimal amount of amboceptor.

governing C′ is either a quite complex one or that there are qualitative or quantitative differences between the sera used.

The rate of rise of the part CD is, except when very small amounts of amboceptor are used, practically independent of the amount of amboceptor present.

In order to determine whether CD continues indefinitely as a straight line or not, curves were run with very small amounts of amboceptor and correspondingly large amounts of complement. Two such curves are shown in the upper half of Fig. 15. The upper curve was made with 0.018 c.c. of heated immune serum (No. 2) as amboceptor, the lower curve with 0.0135 c.c. of the same serum. The complement was increased to five c.c. in each curve.

From these curves it is seen that to the complement curves previously obtained there must be added a fourth part, DE (Fig. 9),

also apparently a straight line. The rate of rise of DE is, at least approximately, independent of the amount of amboceptor present. Whether DE continues indefinitely as a straight line or not has as yet not been determined.

Attempts to express the complete complement curve as a mathematical equation have thus far been unsuccessful.

THE AMBOCEPTOR.

In a similar way curves were plotted showing the change in hemolytic power as the amboceptor varies. To do this, increasing

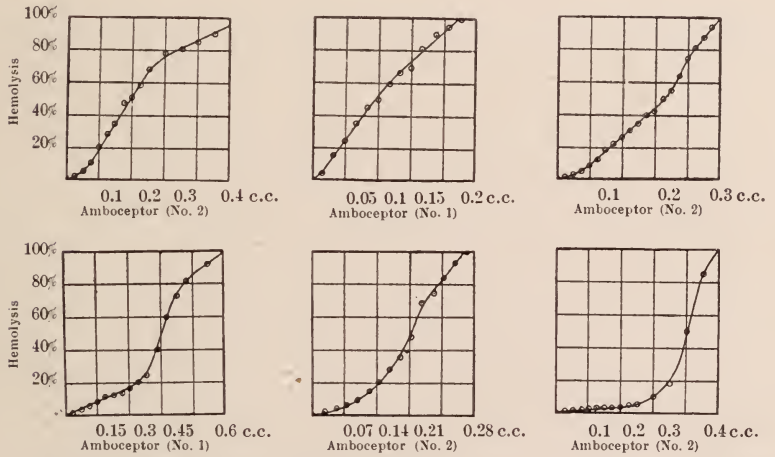


Fig. 10.—Amboceptor curves. Read from above the curves were made with the following amounts of complement: 0.33⅓ c.c., 0.43¾ c.c., 0.20 c.c., 0.26¾ c.c., 0.23⅓ c.c., 0.06¾ c.c.

amounts of amboceptor were added to a series of tubes, the volumes made up to a constant quantity, a constant amount of complement added, and corpuscles exposed to the action of the mixtures. A number of curves obtained in this way are shown in Fig. 10.

To determine the effects of the amount or complement on the nature of these curves, parallel curves were run as before, with the same increasing amounts of amboceptor, but with different constant amounts of complement. A series of such curves is shown in Fig. 11.

Three additional series of parallel amboceptor curves, are shown in Figs. 12, 13 and 14. In Fig. 14 quite small amounts of com-

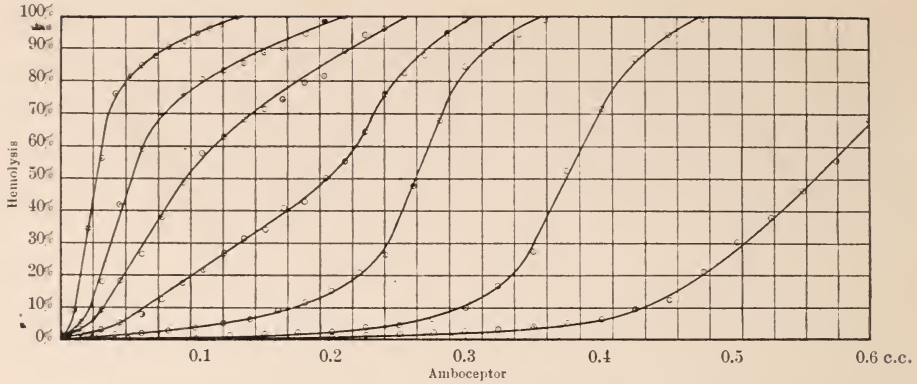


FIG. 11.—Parallel amboceptor curves. (Normal Serum No. 3, Immune Serum No. 2.) Read from below upwards, the curves were made with the following amounts of complement: 0.03⅓ c.c., 0.06⅔ c.c., 0.13⅓ c.c., 0.20 c.c., 0.26⅔ c.c., 0.33⅓ c.c., 0.53⅓ c.c.

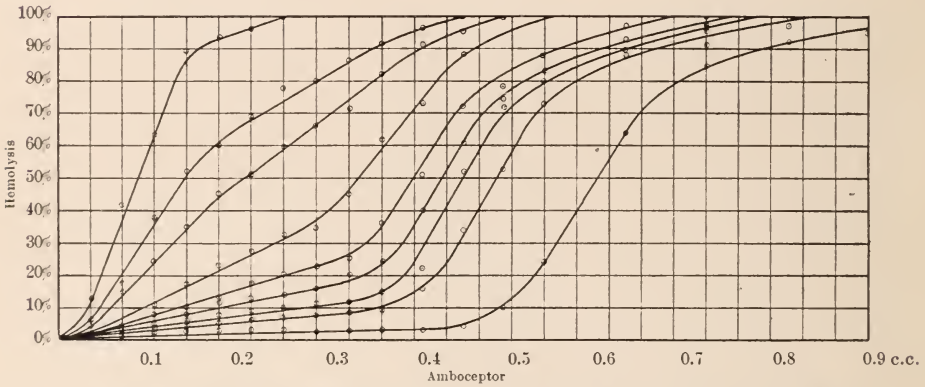


FIG. 12.—Parallel amboceptor curves. (Normal Serum No. 3, Immune Serum No. 1.) Read from below upwards, the curves were made with the following amounts of complement: 0.13⅓ c.c., 0.2 c.c., 0.23⅓ c.c., 0.26⅔ c.c., 0.3 c.c., 0.36⅔ c.c., 4.6⅔ c.c., 0.56⅔ c.c., 0.9 c.c.

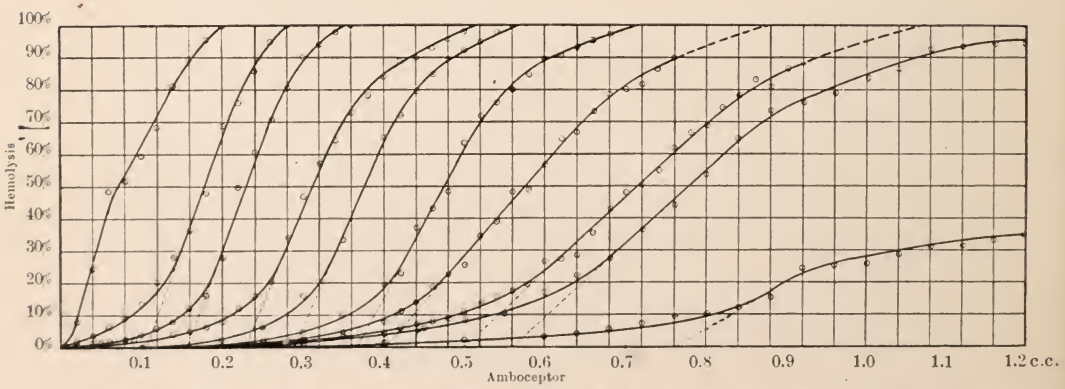


FIG. 13.—Parallel amboceptor curves. (Normal Serum No. 4, Immune Serum No. 2.) Read from below upwards, the curves were made with the following amounts of complement: 0.015 c.c., 0.0225 c.c., 0.03 c.c. 0.04 c.c., 0.05 c.c., 0.06⅔ c.c., 0.10 c.c., 0.16⅔ c.c., 0.23⅓ c.c., 0.36⅔ c.c.

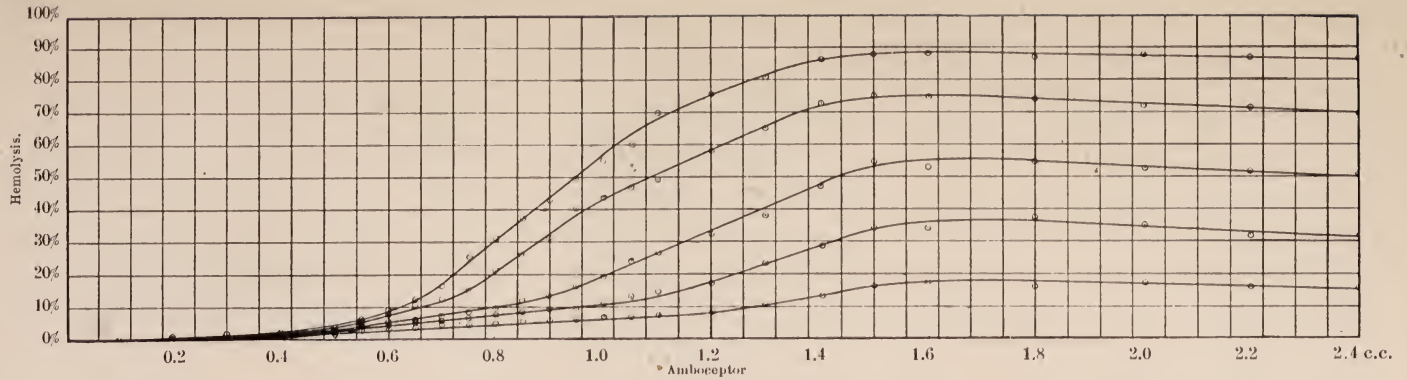


FIG. 14.—Amboceptor curves with minimal amounts of complement. (Normal Serum No. 3, Immune Serum No. 2.) Read from below upwards, the curves were made with the following amounts of complement: 0.01 c.c., 0.0125 c.c., 0.015 c.c., 0.0175 c.c., 0.02 c.c., 0.0225 c.c.

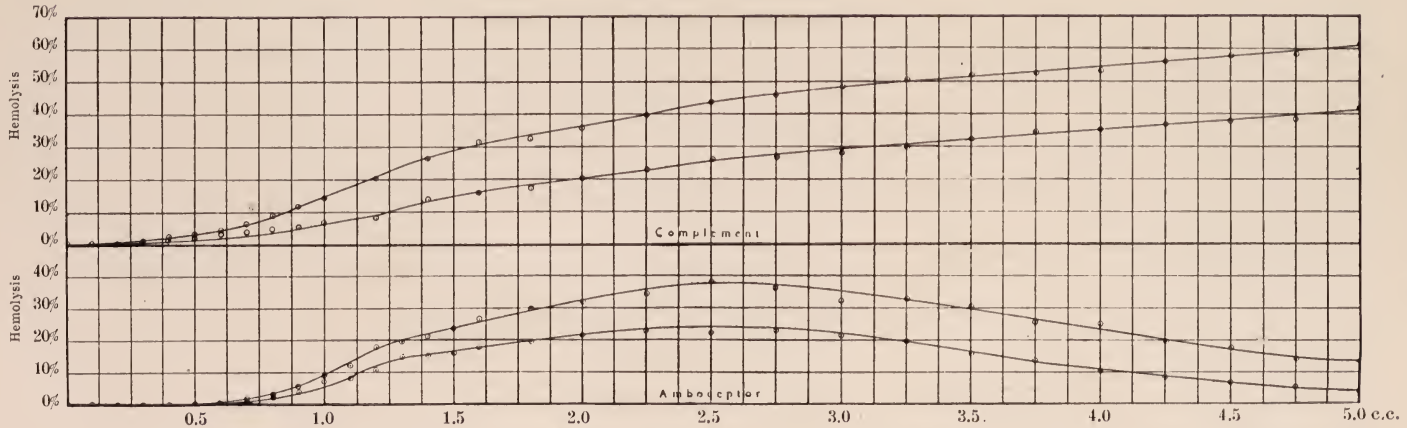


FIG. 15.—Complement and amboceptor curves compared. (Normal Serum No. 4, Immune Serum No. 2.) The two lower curves were made with 0.0135 c.c. and 0.018 c.c. of complement, the two upper with the same amounts of amboceptor. The curves were made on the same day and with the same corpuscles.

plement were used, and correspondingly large amounts of amboceptor. A second set of curves made with very small amounts of complement and still larger amounts of amboceptor is shown in the lower half of Fig. 15.

From these curves it is seen that the typical amboceptor curve (Fig. 16) consists of four parts: an initial part AB, characterized

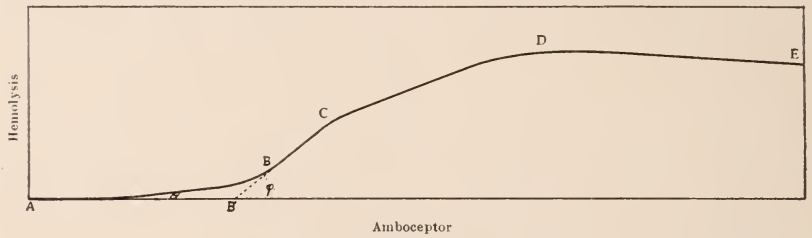


FIG. 16.—The typical amboceptor curve.

by a slow, either uniform or gradually increasing rise (AB apparently differing with the two immune sera used); a second part BC, characterized by a rapid, uniform rise; a third part, CD characterized by a slow, uniform rise; and a fourth part, DE, characterized by a slow, gradually decreasing fall.

The length of the portion AB decreases with increase in the amount of complement used. The quantitative relation between the two is shown in the following table, taken from Fig. 13, from which it is seen that

$$AB' \times \sqrt{\text{Complement}} \text{ not equal to K, a constant.}$$

a relation that might have been expected from a study of complement curves.

Complement	AB'	$\sqrt{\text{Complement}}$	$AB' \times \sqrt{\text{Complement}}$
0.01½	19.1	.123	**2.35**
0.02¼	14.0	.146	**2.04**
0.03	12.5	.173	**2.16**
0.04	10.1	.200	**2.02**
0.05	9.2	.224	**2.06**
0.06⅔	7.2	.258	**1.86**
0 10	5.7	.317	**1.80**
0.16⅔	3.9	.411	**1.60**
0.23⅔	2.7	.472	**1.27**

The rate of rise of that part of AB that is represented by a straight line, increases with increase in the amount of complement present. The relation between the two is shown in the following table, taken from Fig. 12, from which it is evident that

$$\frac{\tan \theta}{\text{Complement}} \text{ not equal to K}$$

a relation that might have been expected from a study of complement curves.

Complement	θ	$\tan \theta$	$\frac{\tan \theta}{\text{Complement}}$
0.1⅓	2°	0.03	**0.2**
0.2	4°	0.09	**0.5**
0.2⅓	7° 30′	0.13	**0.6**
0.2⅔	11°	0.20	**0.8**
0.3	17°	0.30	**1.0**
0.3⅔	24°	0.44	**1.2**
0.4⅔	42°	0.90	**1.9**
0.5⅔	52° 30′	1.30	**2.3**
0.9	69°	2.60	**2.9**

The rate of rise of the part BC increases, when small quantities of complement are used (Figs. 13 and 14), with increase in complement. With larger amounts of complement (Figs. 12 and 13) the rate is practically independent of the amount of complement present. The relation between the two is shown in the following table, taken from Fig. 13, from which it is evident that

$$\frac{\tan \phi}{\text{Complement}} \text{ not equal to K}$$

a relation that might have been expected from a study of complement curves.

Complement	ϕ	$\tan \phi$	$\frac{\tan \phi}{\text{Complement}}$
0.01½	29°	0.55	**37**
0.02¼	43°	0.92	**41**
0.03	44°	0.96	**32**
0.04	48° 30′	1.12	**28**
0.05	61°	1.80	**36**
0.06⅔	66°	2.27	**34**
0.10	67°	2.33	**23**
0.16⅔	69° 30′	2.67	**16**
0.23⅔	72°	3.00	**13**
0.36⅔	74° 30′	3.60	**10**

The height to which BC rises increases, when small amounts of complement are used, with increase in complement. With larger amounts of complement it is practically constant. No mathematical expression has yet been found for this height. The length BC decreases when large amounts of complement are used, due to the increase in the height of B, until, with very large amounts, BC = O.

The rate of rise of the part CD (Figs. 14 and 15) is practically independent of the amount of complement present. The height to which D rises (maximum hemolysis) bears no simple relation to the amount of complement present, the relation between the two being shown in the following table:

Complement	Maximum Hemolysis
.01. c.c.	18%
.01¼	27
.01½	55
.01¾	76
.02	88
.02¼	92

The rate of fall of DE (Figs. 14 and 15) is very nearly independent of the amount of complement present.

The difference between the amboceptor and complement curves is plainly shown in Fig. 15, the curves of which were made with the same sera, the same corpuscles, and on the same day. In this series the routine technique was varied by making up the volumes in the various tubes with $\frac{m}{8}$ NaCl in place of the dilute heated normal serum heretofore used. This change, apparently, made no difference in the general nature of the curves.

Attempts to express the complete amboceptor curve as a mathematical equation have thus far been unsuccessful.

THE WHOLE SERUM.

Experiments were also undertaken to determine the effect on hemolytic power of varying both complement and amboceptor simultaneously. To do this, increasing amounts of immune serum or of an immune serum mixture were placed in a series of

hemolytic tubes, the volumes made up to a constant quantity, corpuscles added, and curves plotted as before. A curve obtained in this way is shown in Fig. 17.

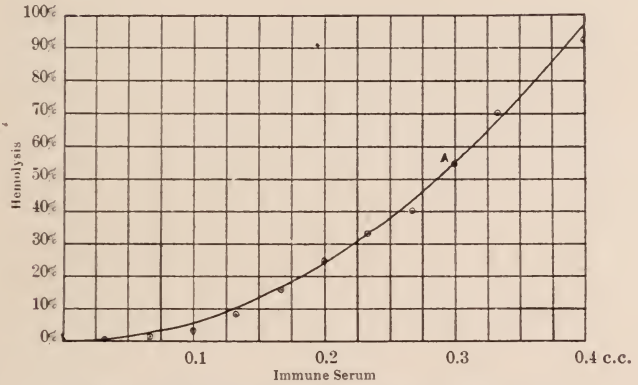


Fig. 17.—The curve of squares. (Immune Serum No. 1.)

This curve was apparently so nearly the parabola determined by the equation

$$\text{Hemolysis} = K\,(\text{Serum})^2$$

that in drawing it an accurately determined point, A, was selected as a point of true observation and the curve calculated mathematically from it. The agreement between the observed hemolysis and the hemolysis calculated from this assumption is shown below:

Serum	Hemolysis (obs.)	Hemolysis (calc.)
c.c.	%	%
0.00	0	0
0.03⅓	0+	1.5
0.06⅔	1.5	3
0.10	4−	6
0.13⅓	8.5	11
0.16⅔	16	17
0.20	25	24
0.23⅓	33	33
0.26⅔	40	43
0.30	55	[55]
0.33⅓	70	68
0.40	92.5	97

A second curve obtained with the same serum two weeks later is shown in Fig. 18. The relation between the observed and calculated hemolysis in this curve is shown below:

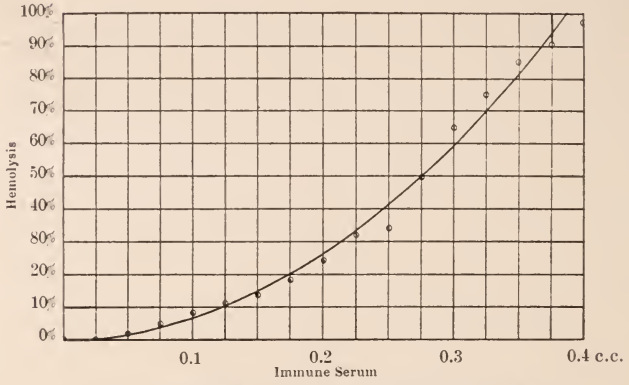


FIG. 18.—The curve of squares. (Immune Serum No. 1.)

Serum c.c.	Hemolysis (obs.) %	Hemolysis (calc.) %
0.00	0	0
0.025	0+	0.4
0.05	2.5	1.6
0.075	5	3.8
0.10	8.5	6.6
0.125	11.25	10.3
0.15	13.75	15
0.175	18.5	20
0.20	24	26.5
0.225	33	33
0.25	34	41
0.275	50	[50]
0.30	65	60
0.325	75	70
0.35	85	81
0.375	90	93
0.40	95	106

From this agreement a law might readily have been deduced that, the volume and amount of corpuscles remaining constant, the amount of hemolysis increases as the square of the amount of serum used, were it not for the fact that the serum from the second immune goat gave a totally different curve (Fig. 19).

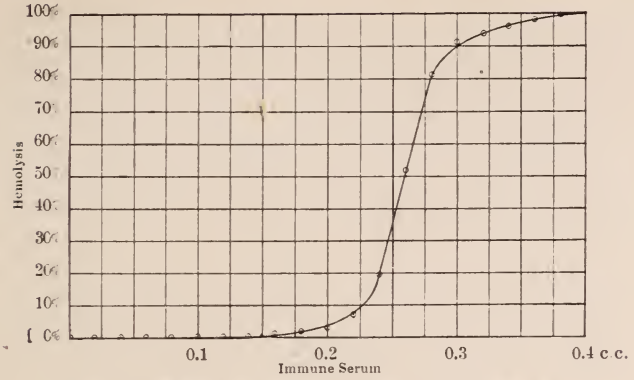


Fig. 19.—Whole-serum curve. (Immume Serum No. 2.)

Three months later, after eight additional injections, a curve similar to this was obtained from the goat that had formerly given the simpler result (Fig. 20), at which time the second immune goat gave an even more striking dissimilar curve (Fig. 21).

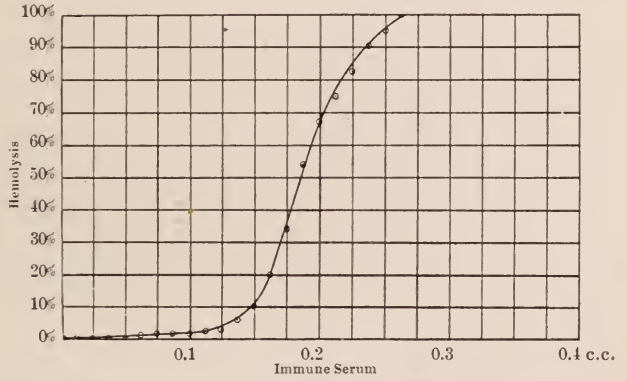


Fig. 20.—Whole-serum curve. (Immune Serum No. 1, three months later.)

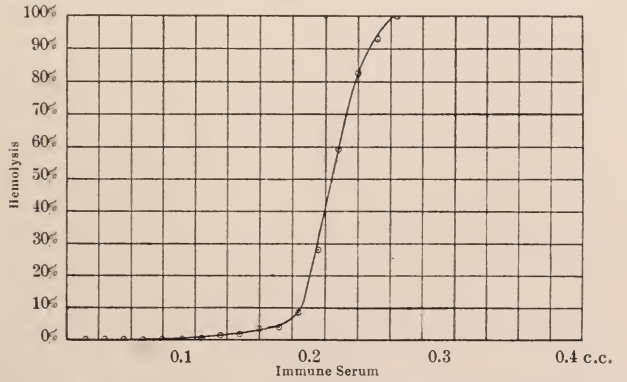


Fig. 21.—Whole-serum curve. (Immune Serum No. 2, three months later.)

It was suspected from this change that the apparent parabolas obtained above were but special cases of more complex curves. A re-examination of data showed this in reality to be the case, the true hemolytic curve being, even in these instances, a curve of double curvature. The relation between this true curve and the calculated parabola is shown in Fig. 22, which was made from the data of Fig. 18.

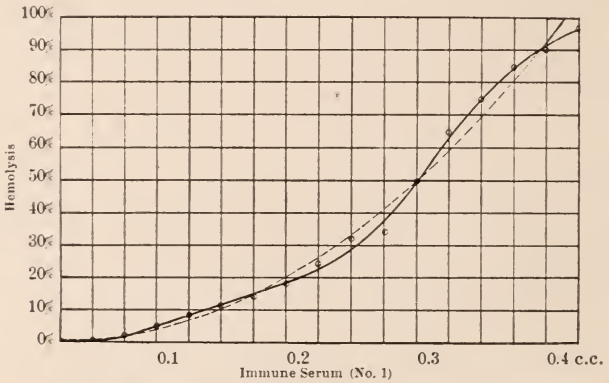


FIG. 22.—Whole-serum curve and curve of squares compared.

As the curve changed with repeated injections, it was thought that its nature might depend on the relative amount of complement and amboceptor present. To test this hypothesis, curves were plotted with various mixtures of normal serum (complement) and heated immune serum (amboceptor). A number of these are shown in Fig. 23.

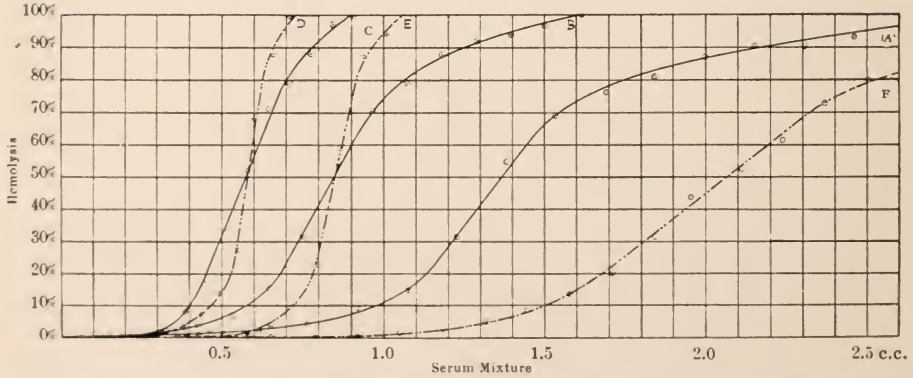


FIG. 23.—Whole-serum curves. (Immune Serum No. 1, Normal Serum No. 4.) Read in alphabetic order the curves were made with the following approximate amboceptor-complement ratios: 1:40, 1:13, 1:3, 1:1, 5:1, 20:1.

By interpolating a curve between A and B a curve is obtained that approximates a parabola. This curve would correspond to an approximate amboceptor-complement ratio 1 : 20 (each measured in the arbitrary units). A curve interpolated between B and C would approximate Fig. 19, and would correspond roughly to an amboceptor-complement ratio 1 : 8. A curve between D and E would correspond roughly to Fig. 20, and to an approximate ratio 3 : 1.

Attempt was made to reproduce the parabola with an artificial amboceptor-complement mixture. This was successful (Fig. 24). The agreement between the calculated and observed hemolysis in this curve is shown below:

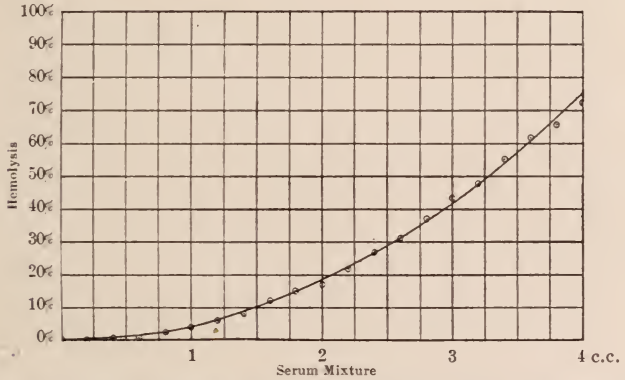


Fig. 24.—Coincident whole-serum curve and curve of squares. The curve was made with a mixture of heated immune serum No. 2 and normal serum No. 3, in the amboceptor-complement ratio 1:200.

Serum c.c.	Hemolysis (obs.) %	Hemolysis (calc.) %
0.0	0	0
0.2	0	0.2
0.4	0.25	0.7
0.6	0.5	1.7
0.8	2.5	3
1.0	4	4.7
1.2	6	6.7
1.4	8	9.2
1.6	12	12
1.8	15	15.2
2.0	17	18.8
2.2	22	22.7

Serum c.c.	Hemolysis (obs.) %	Hemolysis (calc.) %
2.4	27	27
2.6	31	31.7
2.8	37	36.7
3.0	43	42.2
3.2	48	[48]
3.4	55	53.6
3.6	62	60.8
3.8	66	67.7
4.0	72	75

The typical whole-serum curve (Fig. 25) can be divided into four parts: an initial part, AB, characterized by no hemolysis; a second part, BC, characterized by a gradually increasing rise; a

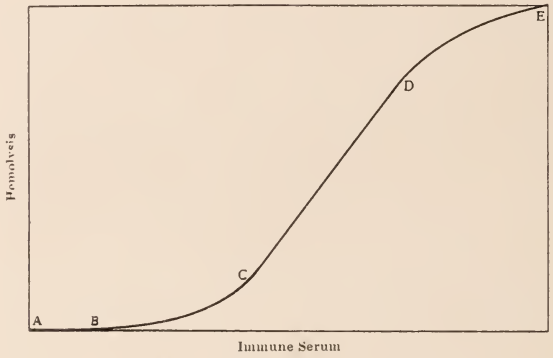


FIG. 25.—The typical whole-serum curve.

third part, CD, characterized by a rapid, uniform rise; and a fourth part, DE, characterized by a slow, gradually decreasing rise.

The length AB is roughly proportional to the amount of amboceptor present, the relation between the two being shown by the following table, taken from Fig. 23:

Amboceptor	AB
0.075 c.c.	1—
0.15	1.5—
0.30	2—
0.60	2.5
1.20	4.5
2.50	9

The rate of rise of the part CD is greatest when the amboceptor and complement are approximately equal in amount, and decreases as either predominates. The height to which CD rises is, in the curves thus far obtained, apparently independent of the relative amounts of complement and amboceptor used. No simple mathematical relations have been discovered.

Attempts to express the whole-serum curve as a mathematical equation have thus far been unsuccessful.

THE CORPUSCLES.

Experiments were also undertaken to determine the effect of changes in the number of corpuscles on the amount of hemolysis

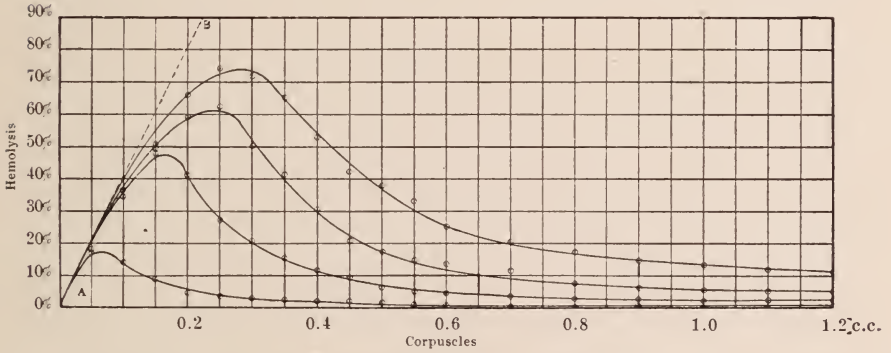


Fig. 26.—The corpuscle curve. Read from below upwards the curves were made with the following amounts of immune serum No. 2: 0.13⅓ c.c., 0.16⅔ c.c., 0.20 c.c., 0.23⅓ c.c.

produced. To determine this, increasing amounts of corpuscles were added to equal amounts of immune serum or to an immune serum mixture, and the hemolysis estimated colorometrically, as percentages of total hemolysis when 0.25 c.c. of corpuscles are used.

A number of curves obtained in this way are shown in Fig. 26. From these it is seen that as the amount of corpuscles increase, the amount of hemoglobin liberated increases, at first rapidly, but soon falls short of the tint that would have been obtained were all the corpuscles present dissolved. The curve of complete lysis is represented by AB. The amount of hemoglobin liberated soon reaches a maximum, and then rapidly decreases, presumably eventually to zero.

With the larger quantities of serum used, the maximum tint was roughly proportional to the amount of serum present, a relation, however, that did not hold when smaller amounts of serum were present. The available data is too meager to determine the exact ralation between the two. The relation in Fig. 26 is shown below:

Serum	Maximum Hemolysis
0.13⅓ c.c.	18%
0.16⅔	48
0.20	62
0.23⅓	74

No mathematical expression for the corpuscle curve as a whole has as yet been obtained.

CONCLUSION.

The curves here reported are part of work preliminary to the study of neutralization curves for hemolytic sera. They furnish, however, data by means of which some of the physico-chemical laws proposed by Arrhenius can be tested. A mathematical analysis of these data with this object in view will be the subject of a separate report.

THE ABSORPTION OF HEMOLYTIC AMBOCEPTOR.*

WILFRED H. MANWARING.

(*From the Pathological Laboratory of the University of Chicago.*)

AS PART of a somewhat extended study of the physical chemistry of hemolytic serum, it was necessary to determine the amount of hemolytic amboceptor absorbed by corpuscles. The result of this determination being at variance with results recently published by Arrhenius[1] its early publication is desirable. A more extended study of absorption phenomena will be reported later.

The serum used in this work was that of goats immunized against sheep corpuscles. The hemolytic experiments were performed in accordance with the technique adopted in this laboratory and described elsewhere in this issue.[2]

To determine the amount of amboceptor absorbed by corpuscles, varying amounts of heated hemolytic serum (amboceptor) were placed in large centrifuge tubes, the volumes made up to a constant (10 c.c.) with $\frac{m}{8}$ NaCl-solution and a uniform amount of washed sheep corpuscles (seven c.c. of a seven per cent suspension of corpuscles No. 1) added to each. The tubes were shaken, incubated at 37.5°C. for three hours and placed in the ice-chest over night, exactly as in the routine hemolytic experiment. The next morning the fluid in each tube was again mixed to insure its uniformity throughout, the corpuscles thrown down by centrifugation, and accurately measured quantities of the supernatant liquid removed for analysis.

The analysis was performed by means of the amboceptor curve.[3] This is the curve showing the changes in hemolytic power as the amboceptor increases in amount, and is obtained by exposing corpuscles to increasing quantities of amboceptor, in

*The first half of this paper was presented before the American Association of Pathologists and Bacteriologists, April 22, 1905; the second half before the Chicago Pathological Society, June 12, 1905. Received for publication May 29, 1905.

[1] *Arbeiten aus dem Kaiserlichen Gesundheitsamte*, Bd. XX, p. 559.

[2] See p. 462.

[3] See p. 471.

the presence of a constant amount of complement. The fluid to be analyzed was added to the same amount of complement, and the resulting hemolysis compared with readings on this curve.

Knowing the amount of amboceptor originally added to the corpuscles, the volume removed for analysis, and the amount of amboceptor found in this volume, the loss of amboceptor, or the amboceptor absorbed by the corpuscles can be readily calculated. A series of results obtained in this way are shown in Table 1, and represented graphically in Fig. 1. The small figure in the upper part of Fig. 1 is the amboceptor curve used in the analysis.

TABLE 1.

ABSORPTION OF AMBOCEPTOR NO. 1.

Recorded amounts of heated immune serum No. 1 placed in large centrifuge tubes, volumes made up to 10 c.c. with $\frac{m}{8}$ NaCl, 7 c. c. of a 7 per cent suspension of washed sheep corpuscles No. 1, added to each, recorded amounts removed for analysis. In calculating results allowance was made for volume of corpuscles (0.14 c.c.) and evaporation (0.11 c.c.).

Amboceptor	Amount Analyzed	Hemolysis	Corresponding Amboceptor	Free Amboceptor (Average)	Bound Amboceptor (Average)
5.0 c.c.	1 c.c.	70.0%	0.252 c.c.	**4.22** c.c.	**0.78** c.c.
4.0	1	44.0	0.196	**3.28**	**0.72**
3.5	1	25.0	0.154	**2.66**	**0.84**
	2	95.0	0.320		
2.5	1	10.0	0.100	**1.66**	**0.84**
	2	43.0	0.194		
	3	90.5	0.300		
2.0	1	5.8	0.071	**1.16**	**0.84**
	2	19.0	0.138		
	3	47.5	0.204		
1.5	2	9.0	0.096	**0.79**	**0.71**
	3	19.0	0.138		
1.25	2	7.0	0.080	**0.64**	**0.61**
	3	11.0	0.108		
1.0	3	6.0	0.076	**0.42**	**0.58**
0.8	3	5.0	0.070	**0.39**	**0.41**
0.6	3	4.0	0.052	**0.29**	**0.31**
0.4	3	4 0–	0.048	**0.27**	**0.13**
0.2	3	3.0	0.030	**0.17**	**0.03**

In a recent publication[1] Arrhenius claims that this absorption takes place in accordance with certain well-established physico-chemical laws, that, in brief, the amboceptor divides itself between corpuscles and surrounding fluid exactly as a dissolved substance divides itself between two immiscible solvents.

[1] *Loc. cit.*

To illustrate, if benzene and water are in contact and a substance soluble in both is added to one of them, the substance will divide itself between the two solvents in the ratio of its solubilities in the two fluids, provided, of course, no chemical change takes

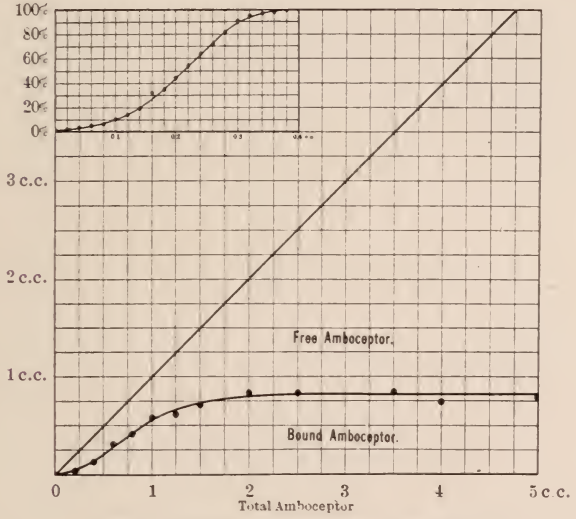


FIG. 1.—The absorption curve. (See Table 1.)

place in either liquid. Thus, if its solubility in water is S grams per liter, and in benzene S' grams, division will take place so that

$$\frac{\text{Concentration* in water}}{\text{Concentration in benzine}} = \frac{S}{S'} = \text{K, a constant.}$$

To this constant, K, the name **Partition Coefficient**, or **Distribution Coefficient**, has been given.

In many cases, however, solution is accompanied by chemical change in the dissolved substance. Thus, benzoic acid dissolved in water has the formula C_6H_5COOH, but on dissolving it in benzine it polymerises to form $(C_6H_5COOH)_2$. For such cases it can be deduced theoretically and verified experimentally[1] that division takes place in accordance with the more complex formula

$$\frac{\text{Concentration in water}}{\sqrt{\text{Concentration in benzene}}} = \text{K.}$$

*Number of gram-molecules per liter.

[1] NERNST, *Theoretical Chemistry*, second English edition (1904), p. 486.

Similarly, if the dissolved substance should exist in benzene as a polymer of three molecules, division would take place so that

$$\frac{\text{Concentration in wator}}{\sqrt[3]{\text{Concentration in benzene}}} = \text{K}.$$

A somwhat complex example, but one that illustrates closely the method of absorption claimed for amboceptor and agglutinins, is afforded by assuming that in one of the solvents (water), the substance exists as a polymer of three molecules, while in the other (benzene), it exists as a polymer of two. Division would then take place in accordance with the formula

$$\frac{\sqrt[3]{\text{Concentration in water}}}{\sqrt[2]{\text{Concentration in benzene}}} = \text{K}.$$

This, according to Arrhenius, is the equation governing the absorption of hemolytic amboceptor; amboceptor dividing itself between corpuscles and liquid so that

$$\frac{\sqrt[3]{\text{Concentration in liquid}}}{\sqrt[2]{\text{Concentration in corpuscles}}} = \text{K}.$$

Using brackets to denote concentration, this may be expressed

$$\frac{[\text{Free amboceptor}]^{\frac{1}{3}}}{[\text{Bound amboceptor}]^{\frac{1}{2}}} = \text{K},$$

and may be simplified to read

$$\frac{[\text{Free amboceptor}]^{2}}{[\text{Bound amboceptor}]^{3}} = \text{K}^{6} = K.$$

Applying this conception to the data obtained above, and calculating K', a multiple of K obtained by omitting to divide by volumes—the volumes are constant throughout and this omission introduces no error—there are obtained values recorded in Table 2. It is seen that K' is not a constant, and that the absorption does not follow the physico-chemical law proposed.

Comparison between the observed absorption and the theoretical absorption of Arrhenius can best be made by calculating the bound amboceptor under the assumption that K' is constant. Table 3 gives such a comparison, the bound amboceptor being

calculated from the observed free ambocepter by means of the value $K' = 2$. Other values of K' give even more strikingly dissimilar results.

TABLE 2.

PARTITION COEFFICIENT.

Free Amboceptor	Bound Amboceptor	K'
4.22 c.c.	0.78 c.c.	37.5
3.28	0.72	28.8
2.66	0.84	11.0
1.66	0.84	4.7
1.16	0.84	2.3
0.79	0.71	1.7
0.64	0.61	1.9
0.42	0.58	0.9
0.39	0.41	2.2
0.29	0.31	2.8
0.27	0.13	33.2
0.17	0.03	1070.0

TABLE 3.

OBSERVED ABSORPTION AND CALCULATED ABSORPTION COMPARED.

Bound amboceptor calculated from the value $K' = 2$.

Free Amboceptor	Bound Amboceptor (Observed)	Bound Amboceptor (Calculated)
4.22 c.c.	0.78 c.c.	2.07 c.c.
3.28	0.72	1.75
2.66	0.84	1.52
1.66	0.84	1.11
1.16	0.84	0.88
0.79	0.71	0.68
0.64	0.61	0.60
0.42	0.58	0.44
0.39	0.41	0.42
0.29	0.31	0.35
0.27	0.13	0.33
0.17	0.03	0.24

The same comparison is shown in Fig. 2, in which the heavy line, AB, represents the observed absorption, and the dotted line, AC, the calculated absorption. The curves agree roughly only when small amounts of amboceptor are used. With large amounts they diverge widely.

In repeating this experiment with immune serum No. 2, and increasing the amount of amboceptor to 10 c.c., a phenomenon

was observed that throws doubt on the experimental method and strikingly illustrates the difficulty of attempting to apply elementary physico-chemical laws to phenomena of this nature, before all factors entering into the phenomena are known. In this experiment, when large quantities of serum were used, apparently as much amboceptor was found in the fluid as was added to it originally, and with still larger amounts, the paradoxical result was

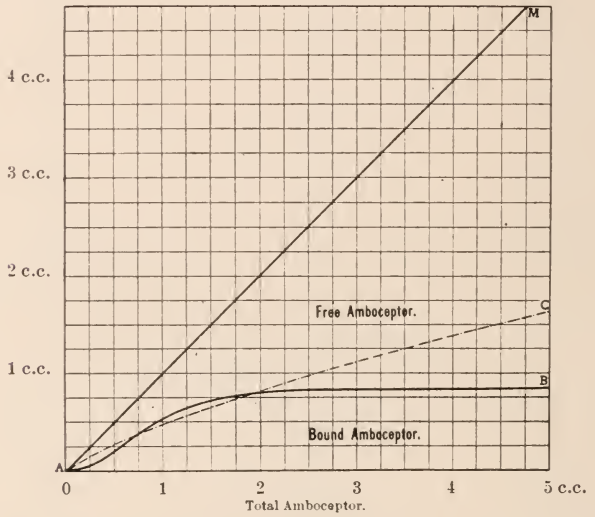


FIG. 2.—Absorption curve and physico-chemical curve compared. AB = observed absorption; AC = calculated absorption from the value K = 2.

obtained that apparently more amboceptor was recovered than was put there in the first place. The data from this experiment are shown in Table 4, and represented graphically in Fig. 3.

An even more striking curve was obtained by using large amounts of immune serum No. 1. This curve is shown in Fig. 4.

On examining the data in Table 4 a very suggestive, though at first glance not a striking fact is evident, that of non-agreement of duplicates. (See Calculated Free Amboceptor.) When equal volumes of serum are used for analysis, duplicate analyses agree within the limits of experimental error. But when unequal volumes are used, the duplicates in all cases differ, the difference following the uniform rule that the smaller amount gives the higher result.

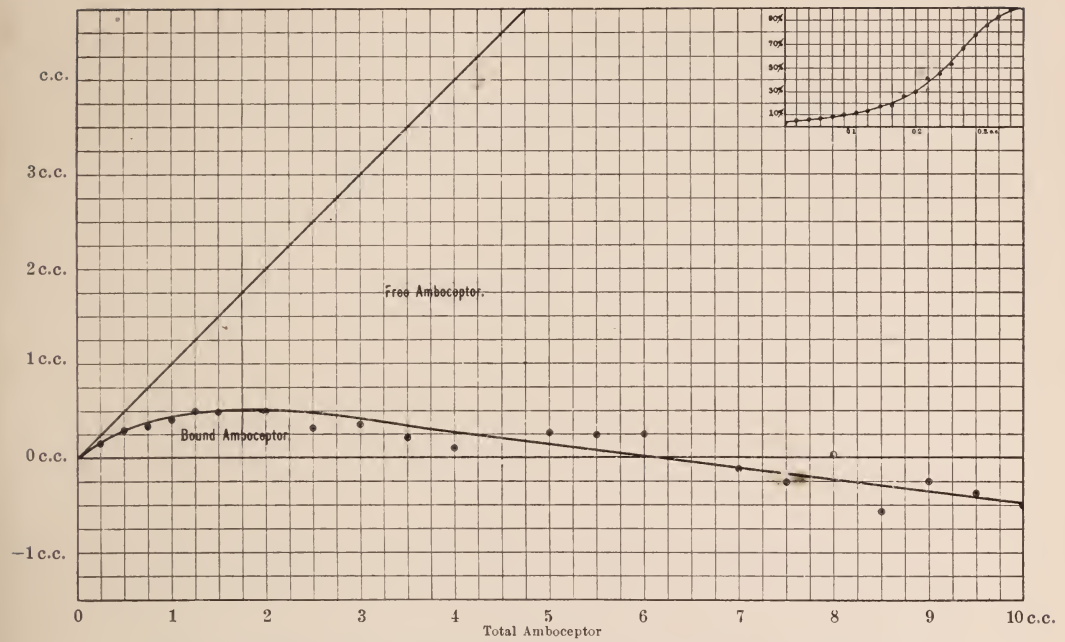


FIG. 3.—Absorption curve with large amounts of serum. (Amboceptor No. 2, see Table 4.)

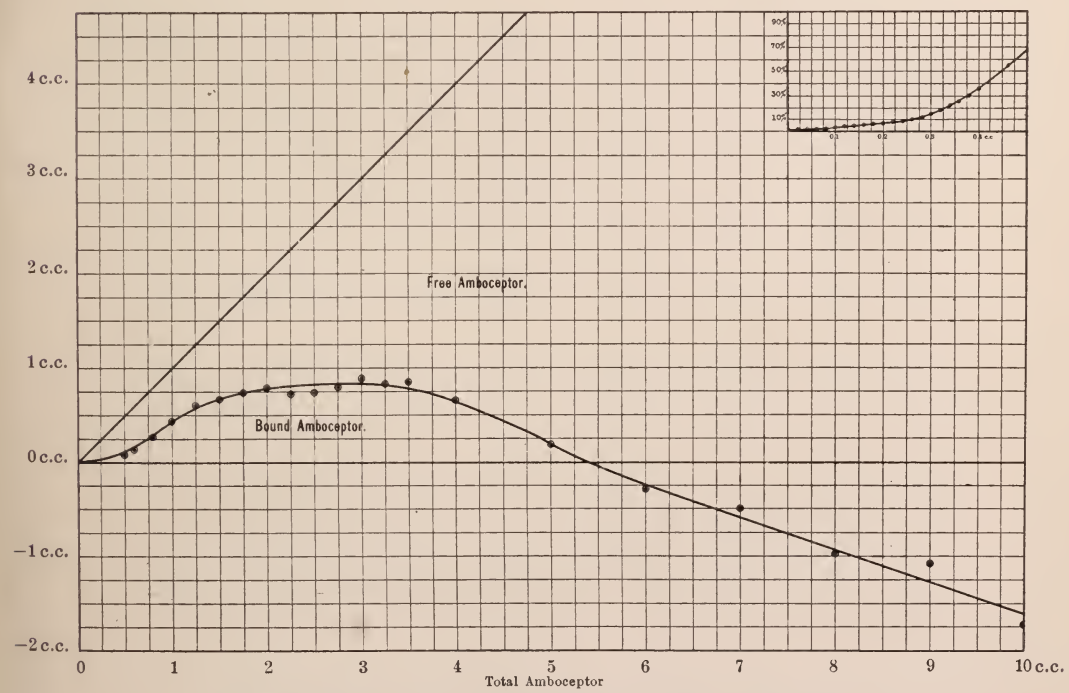


Fig. 4.—Absorption curve with large amounts of serum. (Amboceptor No. 1.)

TABLE 4.

ABSORPTION OF AMBOCEPTOR NO. 2. CORPUSCLES NO. 1.

AMBOCEPTOR	AMOUNT ANALYZED	HEMOLYSIS	CORRESPONDING AMBOCEPTOR	FREE AMBOCEPTOR		BOUND AMBOCEPTOR	K
				Calculated	Average		
10 c.c. ..	0.25 c.c.	20.5%	0.162 c.c.	10.86	10.48 c.c.	−0.48 c.c,	−993
	0.30	30.0	0.194	10.80			
	0.35	33.8	0.204	9.78			
9.5 ..	0.275	24.2	0.176	10.69	9.87	−0.37	−1923
	0.325	30.4	0.194	9.50			
	0.375	35.9	0.211	9.41			
9.0 ..	0.30	24.0	0.175	9.75	9.25	−0.25	−5351
	0.35	29.0	0.191	9.12			
	0.40	36.4	0.212	8.87			
8.5 ..	0.30	23.5	0.173	9.65	9.10	−0.60	−383
	0.35	29.3	0.191	9.13			
	0.40	33.5	0.203	8.53			
8.0 ..	0.30	17.5	0.148	8.24	8.02	−0.02	−6386
	0.35	22.8	0.170	8.14			
	0.40	29.0	0.191	7.69			
7.5 ..	0.35	21.8	0.167	7.97	7.75	−0.25	−3844
	0.40	27.3	0.185	7.72			
	0.45	33.5	0.203	7.57			
7.0 ..	0.40	24.0	0.175	7.32	7.10	−0.10	−50410
	0.45	28.8	0.189	7.04			
	0.50	34.5	0.207	6.94			
6.0 ..	0.4	16.0	0.140	5.88	5.74	0.26	1875
	0.5	23.5	0.173	5.80			
	0.6	30.0	0.198	5.53			
5.5 ..	0.5	21.0	0.164	5.49	5.27	0.23	2282
	0.6	29.3	0.191	5.33			
	0.7	35.0	0.208	4.98			
5.0 ..	0.6	23.8	0.174	4.84	4.74	0.26	1278
	0.7	33.5	0.203	4.88			
	0.8	37.5	0.214	4.49			
4.5 ..	0.7	26.0	0.182	4.36	4.14	0.26	975
	0.75	27.5	0.185	4.14			
	0.9	36.4	0.211	3.92			
4.0 ..	0.7	22.5	0.169	4.06	3.92	0.08	30010
	0.8	30.8	0.196	4.02			
	1.0	40.0	0.220	3.67			
3.5 ..	0.8	24.8	0.178	3.74	3.31	0.19	1597
	1.0	27.5	0.185	3.10			
	1.2	40.5	0.221	3.08			
3.0 ..	1.0	21.0	0.164	2.75	2.65	0.35	160
	1.2	31.0	0.196	2.73			
	1.4	36.3	0.212	2.47			
2.5 ..	1.5	26.5	0.182	2.03	1.94	0.31	126
	1.75	32.5	0.200	1.91			
	2.0	42.0	0.225	1.89			
2.0 ..	2.0	29.0	0.189	1.69	1.49	0.51	17
	2.5	43.0	0.227	1.53			
	3.0	52.5	0.247	1.37			
1.5 ..	3.0	31.1	0.198	1.11	1.01	0.49	9
	3.5	34.5	0.207	0.99			
	4.0	41.3	0.223	0.94			

TABLE 4—*Continued.*

AMBOCEPTOR	AMOUNT ANALYZED	HEMOLYSIS	CORRESPONDING AMBOCEPTOR	FREE AMBOCEPTOR		BOUND AMBOCEPTOR	K'
				Calculated	Average		
1.25c.c..	3.0 c.c.	18.0	0.151	0.84	0.78	0.47	5.9
	3.5	22.0	0.167	0.80			
	4.0	23.5	0.173	0.69			
1.0 ..	3.5	13.8	0.124	0.60	0.58	0.42	4.5
	3.75	14.3	0.128	0.57			
	4.0	15.3	0.137	0.57			
0.75 ..	4.0	10.5	0.097	0.41	0.40	0.35	3.7
	4.0	10.0	0.092	0.39			
	4.0	10.3	0.095	0.40			
0.5 ..	4.0	6.5	0.047	0.20	0.20	0.30	1.5
	4.0	6.5	0.047	0.20			
	4.0	6.5	0.047	0.20			
0.25 ..	4.0	4.8	0.018	0.08	0.08	0.17	1.6
	4.0	4.8	0.018	0.08			
	4.0	4.8	0.018	0.08			

From this it was suspected that there were qualitative as well as quantitative changes in the ambocepter and that these qualitative changes might render direct analysis impossible. To test this hypothesis, amboceptor curves were run with serum before and after contact with corpuscles. Two such curves are shown in

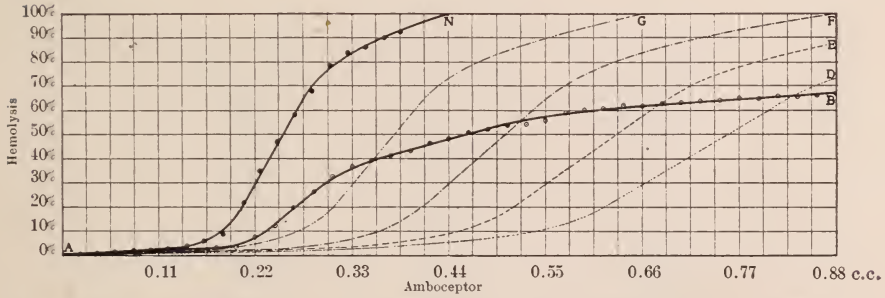


FIG. 5.—Amboceptor curves before and after contact with corpuscles. (Serum No. 1.) AN = normal amboceptor curve. AB = curve after exposure to corpuscles. Dotted curves = theoretical curves representing quantitative changes only (AD = 33⅓%, AE = 40%, AF = 50%, AG = 66⅔%).

Fig. 5, AN being the normal amboceptor curve (before contact) and AB the amboceptor curve after exposure to corpuscles. It is needless to say that the two curves were made with the same serum, the same corpuscles, and on the same day.

The qualitative differences between the two curves are made more evident by drawing theoretical curves under the assumption that there are quantitative changes only in the amboceptor. Four such curves are shown: AD representing the hemolysis under the assumption that the amboceptor is but a third its original strength, AE under the assumption that it is 40 per cent its original strength, AF that it is 50 per cent, and AG that it is $66\frac{2}{3}$ per cent. These curves were obtained by multiplying the abscissas of AN by a constant factor, the ordinates remaining unchanged; but they agree perfectly with experimental curves obtained by using dilute serum.

From Fig. 5 it is seen that if a certain amount of altered serum were used for analysis the percentage of hemolysis would indicate that the amboceptor was over two-thirds its normal strength, if a larger volume were used it would apparently be but half its normal strength, and still larger amounts would give but 40 per cent or even less than $33\frac{1}{3}$ per cent. A series of amboceptor strengths calculated from Fig. 5 is shown in Table 5, from which the impossibility of direct quantitative analysis is evident.

TABLE 5.

AMBOCEPTOR STRENGTH.

Serum No. 1, Corpuscles No. 2 (See Fig. 5).

Volume analyzed	Amboceptor found	Percentage
0.880 c.c.	0.279 c.c.	32%
0.825	0.278	34
0.770	0.277	36
0.715	0.273	38
0.660	0.271	41
0.605	0.269	44
0.550	0.264	48
0.495	0.257	52
0.440	0.249	57
0.385	0.240	62
0.330	0.229	69
0.275	0.209	76
0.220	0.169	77

A second series of curves, made from immune serum No. 2, is shown in Fig. 6, and corresponding strengths of amboceptor in Table 6.

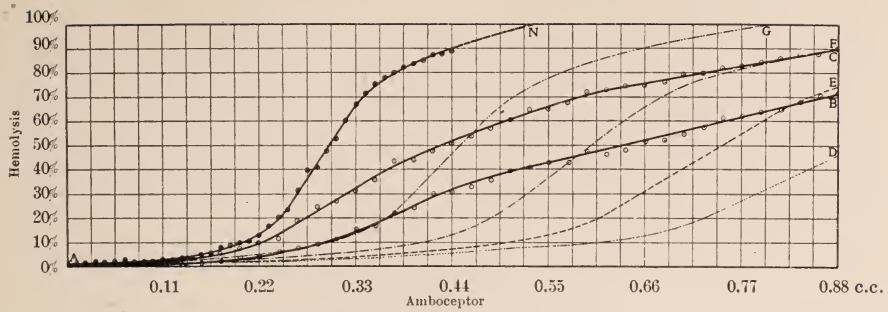


FIG. 6.—Amboceptor curves before and after contact with corpuscles. (Serum No. 2.) AN = normal amboceptor curve. AB and AC = curves after exposure to corpuscles. AC was obtained with half the number of corpuscles used in AB. Dotted curves as in Fig. 5.

TABLE 6.

AMBOCEPTOR STRENGTH.

Serum No. 2, Corpuscles No 2 (See Fig. 6).

	Volume analyzed	Amboceptor found	Percentage
AB.	0.880 c.c.	0.343 c.c.	39%
	0.825	0.330	40
	0.770	0.323	42
	0.715	0.314	44
	0.660	0.303	46
	0.605	0.297	49
	0.550	0.288	53
	0.495	0.279	57
	0.440	0.266	61
	0.385	0.251	65
	0.330	0.224	68
	0.275	0.189	69
AC.	0.880 c.c.	0.440 c.c.	50%
	0.825	0.413	50
	0.770	0.391	51
	0.715	0.370	52
	0.660	0.356	54
	0.605	0.343	57
	0.550	0.330	60
	0.495	0.315	63
	0.440	0.303	69
	0.385	0.288	75
	0.330	0.268	81
	0.275	0.244	89
	0.220	0.198	90

Not only do the curves show qualitative changes, but when serum and corpuscles are put together in certain proportions

the new curve may actually cross the original curve, so that analysis might show more amboceptor than was originally present. Two such curves are shown in Fig. 7, and the corresponding strengths of amboceptor in Table 7. It was the accidental selection of volumes to be analyzed, so as to cause the analysis to fall in the crossed portion of the curve, that gave the paradoxical result noted above.

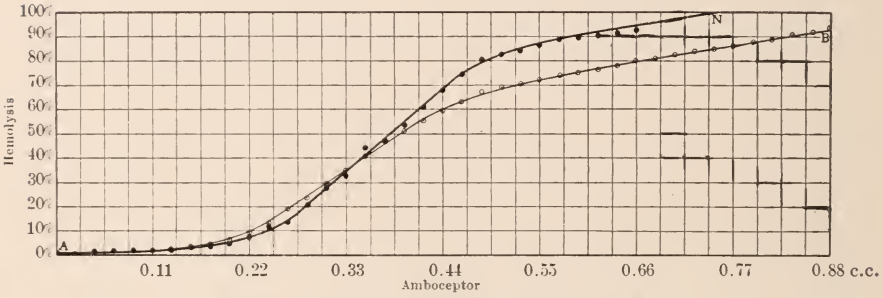


FIG. 7.—Paradoxical amboceptor curves. (Serum No. 2.) AN = normal amboceptor curve. AB = curve after exposure to a minimal number of corpuscles.

TABLE 7.

THE PARADOXICAL AMBOCEPTOR

Serum No. 2, Corpuscles No. 2 (See Fig. 7).

Volume analyzed	Amboceptor found	Percentage
0.880 c.c.	0.627 c.c.	70%
0.825	0.585	71
0.770	0.546	71
0.715	0.515	72
0.660	0.491	74
0.605	0.468	77
0.550	0.449	82
0.495	0.436	88
0.440	0.413	94
0.385	0.374	97
0.330	0.334	101
0.3025	0.310	102
0.275	0.290	106
0.2475	0.270	109
0.220	0.242	110
0.1925	0.216	112
0.165	0.187	113

This crossing of the curves was very marked in a number of experiments with the corpuscles of a certain somewhat anemic sheep (No. 1), but was slight and was obtained only after a number of trials with different amounts, with the corpuscles of a second animal (No. 2). The curves in Fig. 7 were obtained with the second corpuscles.

The nature of the change in the amboceptor and means of overcoming it and thus making analyses possible are now under investigation.

SUMMARY.

1. Using a method of analysis involving direct quantitative comparisons between treated and untreated serum, measurements are obtained not in accord with the physico-chemical law recently proposed by Arrhenius for the absorption of hemolytic amboceptor.

2. Heated hemolytic serum (amboceptor) is so changed by contact with corpuscles that any direct quantitative comparison between it and untreated serum gives erroneous results.

3. This fact makes the value of measurements thus far obtained for the absorption of hemolytic amboceptor exceedingly doubtful, and necessitates a re-examination of data of quantitative work with agglutinins, toxine-antitoxine mixtures, and various hemolysins, to rule out a possible similar source of error.

THE RÔLE OF THE TYPHOID BACILLUS IN THE PULMONARY COMPLICATIONS OF TYPHOID FEVER.*

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The fact that typhoid bacilli are not infrequently found in the lungs during an attack of typhoid fever is well established, but the effect of their presence is still uncertain. Artaud in 1885 was the first to mention the possibility of pulmonary invasion by typhoid bacilli, and he described typhoid-like bacilli in the lungs of two typhoidal patients dying of pulmonary apoplexy. According to Curschmann, lungs, the seat of gangrene, hypostatic splenization, or even lobar pneumonia due to the pneumococcus, undoubtedly may be invaded by typhoid bacilli. When one considers the constancy with which the typhoid bacillus enters the general circulation, and also that the organism has been obtained by a number of observers (Polguère, Montier, Roque and Bancel) from lungs in a state of congestion only then it is clear that the organism quite regularly enters the lungs during typhoid fever. Roque and Bancel obtained the bacillus six times in 16 attempts by lung puncture during life from cases showing only bronchitis or pulmonary congestion. They think that the typhoid bacillus is present in greater numbers in the pulmonary than in the systemic circulation and plays an important rôle in pulmonary complications of typhoid fever.

Although the organism has been recovered repeatedly from the lung, there are in the literature but few cases in which the typhoid bacillus seems surely to have produced a definite pulmonary lesion.

Several observations have been made recently at the Pennsylvania Hospital which throw some light upon the significance of the typhoid bacillus in pulmonary complications of typhoid fever.

* Received for publication April 24, 1905.

Case 1.—N. B., male, white, age 22, laborer, entered the Pennsylvania Hospital (No. 1594) August 19, 1902, service of Dr. Stengel. Patient is an Italian, speaking no English, so no history can be obtained. Temperature 103.2°, pulse 80, respiration 28. The patient is a well built, well-nourished man; tongue coated; heart sounds are clear and distinct; lungs are everywhere resonant and the breath sounds are clear; liver not palpable; spleen is palpable. Abdomen is round and well formed. No tenderness is elicited. There are a few isolated rose colored spots over the abdomen. Leucocytes 1800. Typhoid bacilli agglutinated.

Patient remained in fairly good condition until September 16, when, after having had a slight cough and a slightly blood-streaked sputum for several days, he developed excessive cough, dyspnea, and hemorrhagic expectoration. The sputum is now almost pure blood and every effort to move produces a severe coughing spell. Such examination as can be made discloses subcrepitant and occasional piping rales on the right side. There is some impairment of resonance at the right base posteriorly. Few rales over left side. Heart sounds clear. Temperature rose to 104.6°, dropped in six hours to 98°, then rose on the next day to 105°.

September 17. Loud, high-pitched, squeak, systolic in time and without diastolic element is heard in the region of the apex of the heart. A few pleural friction sounds can also be heard. Temperature irregular, ranging from 105° to 97°.

September 23. Patient had a chill lasting several hours, after which he became steadily worse and died on September 24.

Autopsy (No. 299) by Dr. Longcope.

Anatomical Diagnosis.—Typhoid fever, healing ulcers of the ileum and cecum; thrombosis of branch of right pulmonary artery; abscess of right lung; acute fibrinous pleurisy; acute bronchopneumonia; acute splenic tumor; swelling of mesenteric lymph glands, parenchymatous degeneration of liver and kidneys, and infarct of right kidney.

An abstract from the autopsy notes is as follows: "The right lung is found to be bound to the pleura by fresh fibrinous adhesions, and in separating it from the parietal pleura a cavity is opened from which there escapes gas and a large quantity of dirty yellowish brown pus. The right lung is large and its lobes are bound together by old fibrous adhesions and by a fresh fibrinous exudate. The pleura over the anterior portion of the upper lobe and margin of the middle lobe is smooth and glistening, but posterior to this the surface of the lung is covered with a fine fibrous exudate, sprinkled here and there with small hemorrhages. The entire posterior portion of the lower lobe is found to be converted into an abscess cavity which measures nine cm. in length and five cm. in breadth. This cavity has been opened in removing the lung from the body. The inner walls of the abscess are covered with a grayish green necrotic material and along the walls can be seen irregular projections and villus-like processes. The walls are here and there ribbed with cords of tissue similar to the projections. Covering the lung about the cavity there is a heavy creamy fibrinous exudate which is sometimes two cm. in thickness.

"On sectioning the anterior portion of the lung, numerous reddish gray

ill-defined areas of bronchopneumonic consolidation are found. In the posterior portion of the lung the cut surface is smooth reddish gray and soggy and the tissue completely consolidated. The main artery leading to the lower lobe is plugged by a firm red and gray thrombus. The large bronchi can be followed directly into the abscess cavity, where they sometimes open and are sometimes covered with fibrin and pus. The thrombus can be followed down many branches of the artery to the abscess cavity, and the ends of the vessels are found to form the villous processes described in the wall of the abscess. The bronchi at the root are filled with blood and mucus.

"The left lung is voluminous. Most of the surface is covered by old fibrous adhesions.. The entire lung is semi-solid and feels as if filled with firm irregular nodules. The cut surface shows dark red hemorrhagic consolidations which stand up above the surface and present a striking contrast to the pale crepitant intervening lung tissue. The consolidations are numerous, large and confluent posteriorly, but anteriorly they are more sparsely scattered and smaller. Frequently the hemorrhagic nodules have a yellow center. The vessels are free from thrombi. The bronchi are injected and contain mucus.

"In the ileum about 80 cm. from the ileocecal valve a large, slightly swollen Peyer's patch is found, showing a rounded ulceration 0.5 cm. in diameter at its lower end. Nearing the cecum other ulcers are found, most of which are small and have clean bases. Some are partially covered with irregular extensions of the mucosa over the surface. The margins of some of the larger ulcers are deeply injected and irregular, but usually they are smooth and rounded. A few small ulcers are found in the cecum, but otherwise the large intestine shows no change.

"The spleen is much enlarged and soft. The mesenteric glands, many of which are the size of almonds, are swollen and injected.

"Iliac vessels free from thrombi."

The condition of the other organs is unimportant.

Microscopic examination.—(Zenker's fluid, paraffin, hematoxylin and eosin).

In all sections of the lungs an extensive bronchopneumonia is found principally of a desquamative type. Many alveoli are filled with desquamated epithelial cells, only a few of which are phagocytes. Elsewhere coagulated serum fills the alveoli either alone or together with desquamated epithelial cells, and a few polymorphonuclear leucocytes and red blood corpuscles. In certain foci, especially about the bronchioles, a characteristic exudate of fibrin and leucocytes extends in the alveoli, and sometimes in these areas there are hemorrhages covering the space of several alveoli and destroying the alveolar walls. The center of such foci are usually formed by a bronchus filled with fibrin, leucocytes, and nuclear fragments. The epithelium of the bronchi is desquamated and often the exudate is attached to the wall. The areas of bronchopneumonia appear to surround and extend from the bronchioles.

Sections stained by the Gram-Weigert method, fail to show any bacteria. Sections stained with Loeffler's methylene blue (Flexner's method for typhoid bacilli in tissues) show collections of well stained rod-like bodies, which in places, especially where polymorphonuclear leucocytes are most numerous, are formed into rather loose, well defined masses.

The wall of the abscess is composed entirely of a mass of necrotic material, leucocytes, fibrin, and nuclear fragments. Directly beneath this exudate, the blood vessels are seen to be much engorged, and masses of red blood cells, closely packed and scattered through the tissue, are present. The blood and deeper portion of the exudate show beginning organization. The lung beneath shows extensive desquamation of epithelial cells with here and there areas of exudative pneumonia.

A section stained by Loeffler's methylene blue shows large numbers of typical small rod-shaped bodies, especially in the tissue where necrosis has only partially taken place, in the area showing the hemorrhage. These bodies have exactly the morphology of the typhoid bacillus.

Mesenteric lymph glands show extensive endothelial proliferation.

Intestines show typical typhoid ulcerations.

Liver shows focal necrosis and fatty degeneration: the kidneys parenchymatous degeneration: and the spleen endothelial proliferation.

Bacteriologic examination.—From the abscess of the lung, pleural fluid, left lung, right lung, thrombus in lung, and kidney, B. typhosus was isolated. It was a small, rather slender bacillus, with rounded ends, rather irregular in length, often in threads, which stain fairly well in proportions of 1-2, 1-3, 1-4, 1-6. It was actively motile, did not stain by Gram, gave a positive agglutination with known typhoid serum at 1 to 100 dilution in one hour and grew characteristically in all respects like B. typhosus. Streptococcus pyogenes was isolated from the renal infarct and liver and B. lactis aërogenes from the pulmonary thrombus, kidney, liver, and renal infarction.

Summary.—After being in the hospital with a typical attack of typhoid fever 27 days, the patient suddenly develops the symptoms and signs of lung infarction, after which he passes into a marked toxemic condition and dies eight days later.

At autopsy the main artery leading to the lower lobe of the right lung is found thrombosed, and that lobe is converted almost entirely into an abscess cavity. A bronchopneumonia is found in other portions of the right lung, as well as in the left lung. A pure culture of typhoid bacillus is found in the lung abscess and in both lungs, and bacillus-like bodies alone are seen in sections stained by methylene blue but no bodies resembling organisms are seen in sections stained by the Gram-Weigert method. In the other organs the lesions are typical of typhoid fever.

As no other causative agent is to be found we feel justified in considering the case one of lung abscess due to B. typhosus, consecutive to pulmonary thrombosis.

A somewhat similar case is reported by Flexner and Harris, in which at autopsy were found typhoid fever without intestinal

lesions, typhoid bacteriemia, thrombosis of the main branch of the pulmonary artery supplying the lower lobe of the right lung, gangrene of the lung, perforation of the pleura and pyopneumothorax. The typhoid bacillus was recovered from the consolidated right lung, the spleen, the liver, and the left lung. No other cases of lung abscess or gangrene in typhoid fever with bacteriological reports have been found in the literature.

From the foregoing cases we feel justified in concluding that B. typhosus is capable of causing pulmonary abscess and gangrene in lung tissue, already the seat of hemorrhagic infarction. That the organism may invade an infarcted area of the lung without causing abscess formation is shown by another case where the pulmonary artery was occluded by a thrombus, typhoid bacilli alone being isolated from the infarcted area. In this case there was no clinical evidence of the infarction. Sections through the pulmonary artery at the site of thrombosis stained with methylene blue showed in several places rod-like bodies lying between the thrombus and the wall of the pulmonary artery. No such bodies could be found in the lungs.

Bronchopneumonia is found quite often in the lungs of patients dying of typhoid fever. When the various inflammatory lesions produced by the typhoid bacillus are considered it would seem reasonable to suppose that this organism can produce bronchopneumonia. There are, however, but few observations to support this idea.

Chantemesse and Widal, who were the first to investigate the subject, obtained the organism from the lung in two cases and concluded that certain forms of bronchopneumonia, found in typhoid fever, are to be considered as specific manifestations of the disease. Finkler expressed the opinion that B. typhosus causes splenization and bronchopneumonic solidification of similar appearance, without peribronchial solidification and without a tendency to suppuration. Bruneau considers that bronchopneumonia may be caused by a localization of typhoid bacilli in the bronchi or lungs. Lepine and Lyonnett found acute bronchopneumonia in the lungs of a dog killed 10 days after injection into the trachea of attenuated cultures of B. typhosus.

Several investigators have isolated the bacillus from bronchopneumonic lungs. Gradwohl found it in pure culture in one case. Bancel, who has made an extensive study of the question of the presence of typhoid bacilli in the lung, concludes that in bronchopneumonia other organisms are usually associated. He isolated by lung puncture B. typhosus and a coccus from a bronchopneumonic lung of a typhoid fever patient.

In six autopsies upon typhoid fever cases at the Pennsylvania Hospital in which cultures were taken from bronchopneumonic areas, the typhoid bacillus was found only once, namely, in the case (Case No. 1) reported here, in which it was obtained in pure culture. As no other organism could be found in a careful microscopic search of the tissues, we consider the bronchopneumonia as caused by the typhoid bacillus. In the other five cases the common pyogenic cocci were isolated.

The rôle played by the typhoid bacillus in lobar pneumonia complicating typhoid fever has been much discussed. The earlier writers, Karlinski, Bruneau, and others, considered that lobar pneumonia associated with typhoid fever was always due to a secondary invasion of the pneumococcus. All investigators have found the pneumococcus frequently present in such conditions. It was found three times in the six cases of lobar pneumonia occurring in typhoid fever patients that have recently come to autopsy at the Pennsylvania Hospital. B. typhosus was isolated in but one case, in which both organisms were obtained. Busquet showed that the typhoid bacillus and pneumococcus can exist together in the circulating blood of patients suffering from pneumonia as a complication of typhoid fever. He obtained both organisms simultaneously by blood culture in two cases.

A similar case has been studied at the Pennsylvania Hospital. A patient entered the hospital on the 10th day of a severe typhoid fever. Three days later his temperature became subnormal and remained so until his death, seven days after his admittance to the ward. No definite signs of pneumonia were noted. On the 16th day of his disease 10 c.c. of blood were drawn from his arm vein and distributed among six flasks containing 100 c.c. of broth each, and two flasks containing 100 c.c. of litmus milk each.

Smears made from flasks after 24 hours at 37°C. showed bacilli, and in all but two flasks, one of milk and one of broth, cocci were seen. By transplanting on human blood agar the organisms were found to be typical typhoid bacilli, giving a positive serum reaction up to 1 to 200 dilution in one hour with known typhoid serum, and pneumococci.

In another case of typhoid fever complicated by lobar pneumonia, B. typhosus was obtained in pure culture from the circulating blood on the 17th day of disease, while at autopsy, four days later the pneumococcus alone was obtained from the heart's blood. Here no doubt the organisms were coexistent in the general circulation during life.

Although there is no doubt that the pneumococcus is the usual cause of lobar pneumonitis in typhoid fever, there is considerable evidence that such a lesion may be caused by the typhoid bacillus. Curschmann expressed the opinion that the typhoid bacillus may cause a true lobar pneumonitis, although this must occur very rarely. One of the earliest workers on this subject, Polguère, expressed a similar belief, while other investigators (Karlinski, Fraenkel, Bruneau, Montier, and others) consider a true typho-pneumonia as unproved or even impossible.

Mallory describes a case of fibrinous pneumonia occurring during typhoid fever in which many typhoid-bacillus-like bodies and a few pneumococci were found in sections. From his study of the case he considers that the presence of the typhoid toxin may influence by production of epithelioid cells the character of pneumonia produced by the pneumococcus.

A case of pneumonia, lobar in type, recently came to autopsy at the Pennsylvania Hospital; from the lung was cultivated a pure culture of the B. type of the paratyphoid bacillus. We believe from several points of evidence that this organism was the cause of the pneumonia.

Case 2.—J. N., Italian laborer, age 21 years, entered the service of Dr. Lewis at the Pennsylvania Hospital, April 25, 1904 (No. 298). His illness began five days before with fever, chills, and pain in right side. At time of entrance the patient expectorated bloody-sputum. Physical examination showed typical signs of lobar pneumonia. The leucocytes were however only 5,720. Temperature was somewhat irregular, ranging between 100° and 105°. He died on the eighth day of his disease.

At the autopsy there was found a lobar pneumonitis; acute fibrinous and chronic fibrous pleurisy; acute splenic tumor; cloudy swelling and fatty degeneration of liver; hemorrhages into the gastric mucosa.

The left lung showed no pneumonia, but the right presented an extensive lobar consolidation, crepitus being obtained only along the posterior border. An abstract of the autopsy notes is as follows: Beneath the right pleura small distinct hemorrhages are seen as dark red spots. The cut surface of the right lung is somewhat uneven and has a dark purple color, through which small bronchioles can be seen. From these bronchioles little masses of yellow purulent material can be expressed. Pinkish gray streaks of connective tissue are seen running through the cut surface, which appears much like the cut surface of a congested spleen. The lung presents a hemorrhagic appearance.

Sections of the consolidated part of the right lung, (Zenker's fluid, paraffin, hematoxylin and eosin) present the following picture: The alveolar arrangement of the lung can be made out. Throughout the section there are scattered diffusely many polymorphonuclear leucocytes. These, together with fibrin, coagulated material, a few small round cells, and a few desquamated epithelial cells, usually fill the alveoli. The alveolar capillaries are engorged with blood and stand out prominently. In and directly under the pleura, are large collections of red blood corpuscles. In two or three areas masses of red blood cells fill several adjoining alveoli. In other areas many red blood cells are scattered diffusely through the alveoli. In a few places masses of blood appear to replace the lung tissue. The blood vessels are everywhere filled, and in several of them the blood has a homogeneous conglomerate appearance suggestive of red blood corpuscle thrombi. There is an amount of yellow and black pigment present.

Sections through the same part of the lung as before described, stained with methylene blue according to the method advised by Flexner for staining, typhoid bacilli in the tissues, give the following picture: In a part of the section that contains the largest amount of free blood, there are seen blue staining bacillus-like bodies in considerable numbers. These bodies have the morphology of the typhoid bacillus, and are in places arranged in fairly dense masses. Similar sections stained by Gram stain show nothing resembling bacilli, nor is there anything appearing like the pneumococcus to be seen. The fibrin is well stained but in no place does it resemble the bacillus-like bodies described above, being coarser and in longer strands.

Cultures were made on agar plates at time of autopsy from the heart's blood and consolidated area of the right lung, and a tube of human blood agar was inoculated from the right lung. The plate inoculated from the heart's blood and the blood agar tube remained sterile after incubation for several days at 37° C. The plate inoculated from the consolidated lung after 24 hours incubation at 37°C. showed three rather dense, round, surface colonies one to three mm. in diameter, which are distinctly raised and have a dense center and thin edges, where the colonies are semitranslucent. There are about 15 deep colonies. Coverslips from the surface colonies show somewhat irregular, rather small bacilli. The organism obtained from the lung in pure culture proved to be B. paratyphosus, type B. It was very motile,

fermented mannite readily, but did not ferment lactose or saccharose. It was negative to Gram's stain. Litmus milk was quite blue after four days incubation at 37° C. The organism gave a positive agglutination reaction with serum from a known case of paratyphoid infection, agglutinating in 1 to 100 dilution in one hour.

In coverslips from the lung there was seen a few rather long narrow bacilli, and a small number of cocci in pairs resembling the pneumococcus.

Summary.—Here is a man dying on the eighth day of a disease in which typical physical signs of pneumonia were found. He had bloody expectoration, and a low leucocyte count. At autopsy there is found a hemorrhagic type of lobar pneumonitis. From the lung tissue a pure culture of the B. type of the paratyphoid bacillus is obtained. No other organisms can be found in the tissues, although smears from the lung show pneumococcus-like organisms.

If this case is, as it seems to be, one of lobar pneumonitis, caused by the paratyphoid bacillus, it would encourage the belief that such a lesion may be produced by another organism of the same group and one so much resembling it in its biology and pathogenicity as B. typhosus

There are in the literature a number of cases in which the presence of the typhoid bacillus in consolidated lung tissue was proved, while the search did not reveal the pneumococcus. Foà and Bordoni-Uffreduzzi were the first to report a case in which a pure culture of typhoid bacilli was found in inflammatory pulmonary consolidation of lobar extent. They considered it evidence that such a lesion can be produced by other causes than the micrococcus lanceolatus. Anton and Fütterer isolated the bacillus and an undetermined coccus from the lung of a case in which pneumonia developed during typhoid fever. Bruhl reports a case which showed the signs of typhoid fever only after eight days of what seemed a typical attack of pneumonia. The patient was known to have been previously exposed to typhoid infection. B. typhosus was isolated from the lung, and no pneumococci could be found, even by inoculation of mice. The blood of the patient remained sterile. The author considers the case one of a true typhoid bacillus pneumonia. He also quotes a case of Widal in which B. typhosus was isolated by lung puncture from a case resembling pulmonary tuberculosis. Castaigne reports a similar

case, in which lung puncture made two days after the onset of pneumonia showed a pure culture of typhoid bacilli. Inoculation of mice here also failed to show any pneumococci. Finally, Glacer reported a case of frank pneumonia from which a pure culture of typhoid bacilli was obtained by lung puncture and in the lung tissues of which only bacilli could be found.

The experimental work upon this subject has been too small to be of much service. Lepine and Lyonnet injected attenuated cultures of typhoid bacilli into the tracheas of eight dogs. Three died very soon after the injection, and enormous hyperemia with hemorrhages were found. One dog was killed 10 days after the injection and the lungs showed an acute bronchopneumonia. Four dogs were killed 15, 17, 24 and 30 days after inoculation, and all showed more or less thickening of the alveolar walls. No true lobar pneumonia was found.

The foregoing cases seem to us to be sufficient to warrant the belief that both the typhoid bacillus and the paratyphoid bacillus, type B, are capable of producing pulmonary inflammation lobar in extent.

The hemorrhagic condition of the pulmonary lesion in our case seems especially worthy of emphasis. The appearance of the lung at time of autopsy was obviously different from that of an ordinary lobar pneumonia. Polguère noticed the hemorrhagic condition of pulmonary lesions of typhoid fever and said that bronchopneumonia in typhoid fever could be produced either by the pneumococcus or by the typhoid bacillus; that caused by the latter being the more hemorrhagic. No other investigator of the subject of typhopneumonia has since mentioned this point.

There is considerable evidence that the pulmonary lesions of typhoid fever are especially hemorrhagic, however, from the condition of the sputum in almost all of the cases reported. Anton and Fütterer appear to be the first to note that the sputum is hemorrhagic in certain cases of pneumonia occurring during typhoid fever. These authors state that the patient's mouth was full of blood the day after the onset of pneumonia in the sixth day of typhoid fever. Sahli reports a case of typhoid fever which he considers complicated by pneumonia rather than lung infarction,

in which the sputum was first bloody and then serous. The typhoid bacillus was isolated from the sputum. The sputum is described as hemorrhagic in such cases by Fraenkel, Diendonné, Edel and Glaser. Stühlern, who isolated the typhoid bacillus twice from the sputum of cases with signs of pulmonary consolidation, describes it in both cases as decidedly hemorrhagic. He considers the bacillus a secondary invader of the lung, and questions whether the hemorrhagic condition of the sputum may not be an effect of the presence of the typhoid bacillus in the lung.

Lepine and Lyonnett, in their experimental work, noted the production of enormous hyperemia with hemorrhages in the lungs following the intratracheal injections of attenuated typhoid bacilli.

The fact that the typhoid bacillus may be present in the lungs in the pulmonary complications of typhoid fever, makes it probable that it is not infrequently present also in the sputum under such circumstances. It has been obtained by a number of observers from this source. In 1894 Sahli reported that he obtained the bacillus in pure culture from the sputum. Edel examined the sputum of 11 cases of typhoid fever, 10 of which had bronchitis and one had pneumonia. From the case of pneumonia the typhoid bacillus was obtained. The most extensive study of this question has been made by Jehle. He examined the sputum of 15 cases of typhoid fever and found the bacillus in the sputum nine times, twice in pure culture. He also examined the bronchial secretion at autopsy, and isolated the organism 12 times. This includes five cases of hemorrhagic infiltration of the lung all of which showed the presence of the bacilli in the bronchial secretion. The author concludes that the typhoid bacillus is present regularly in the sputum and bronchial secretions in cases of typhoid fever complicated by pneumonic or bronchopneumonic processes, and that it exists there less regularly in the sputum of cases with simple bronchitis only. Diendonné, Glacer and recently Rau have reported single cases of pneumonia complicating typhoid fever and showing the organism in the sputum. None were in pure culture.

The organism was recently isolated from the sputum of a case of typhoid fever in the Pennsylvania Hospital.

Case 3.—P. B., Italian, age 25, entered Dr. Scott's service on February 26, 1905, on the 10th day of his disease. He complained of headache, malaise and abdominal pain. Rose spots were seen, his spleen was not palpable, and the temperature was 101°. Typhoid bacilli agglutinated by the patient's serum; leucocytes 5,200. On March 10, the 23d day of disease, pain above the liver was complained of. A few fine crepitant rales were heard on inspiration on the right side between the anterior and posterior axillary lines. On the 28th day of disease there was a well marked pneumonia over the entire upper part of the right lobe. Leucocytes 11,100. The next day blood stained sputum was expectorated. On March 18 a blood culture was taken. On the same day cultures of the sputum were made, a technique similar to that of Edel being followed. A fairly large mass of thick tenacious sputum, blood-tinged and deeply rusty throughout, raised by coughing, was collected in a sterile Petri dish and taken directly to the laboratory. The mouth was not previously washed out. In the laboratory the sputum was washed in five successive dishes of sterile salt solution and inoculated on plates of plain agar in two series of three plates each. Plates were also made on glucose agar, in the same dilution as the second in the series. All plates were much overgrown by bacteria except one glucose agar plate, and the third plates of the agar series. From these plates all colonies resembling the typhoid bacillus were transplanted. Only two, one from a glucose agar plate, and one from a plain agar plate, proved to be B. typhosus. The organism is agglutinated by the serum of a known case of typhoid fever in 1 to 200 dilution in one hour. From the blood an organism similar in every respect was obtained in pure culture.

The occurrence of the typhoid bacillus in the sputum shows the importance of taking proper means to prevent infection through this source.

CONCLUSIONS.

The typhoid bacillus not infrequently invades the lung during typhoid fever.

It may invade areas of the lung already the seat of hemorrhagic infarction and there produce abscess formation and gangrene.

The organism may cause bronchopneumonia.

Lobar pneumonia as a complication of typhoid fever is usually due to the pneumococcus. This organism may be present as a general infection in the circulating blood simultaneously with B. typhosus.

It is probable that both B. typhosus and B. paratyphosus, type B, can produce a massive pneumonia, lobar in type. When these organisms are the causative factors, the pneumonia is of a

peculiar hemorrhagic character, which may be recognized clinically from the bloody nature of the sputum.

The typhoid bacillus is not infrequently found in the sputum of typhoid fever patients with pulmonary complications. This fact should be emphasized in order that spread of the disease by this means may be prevented.

This work was done under the direction of Dr. W. T. Longcope, and I wish to express my indebtedness to him for much helpful advice.

REFERENCES.

Anton and Fütterer. *Münch. med. Wchnschr.*, 1888, 35, p. 315.
Artaud, G. *Thèse de Paris*, 1885.
Bancel, L. *Jour. de Phys. et de Path. gen.*, 1903, 5, p. 877; also *Thèse de Lyon*, 1903.
Bruhl. *Gaz. hebd. de med. et de chir.*, 1897, N. S. 2, p. 99.
Bruneau, G. *Thèse de Paris*, 1893.
Busquet. *Revue de med.*, 1902, 2, p. 167.
Castaigne. *Gaz. hebd. de med. et de chir.*, 1897, N. S. 2, p. 649.
Chantemesse and Widal. *Archiv. de phys. normale et path.*, 1887, 9, p. 245.
Curschman, H. *Nothnagel's Encyclopedia of Practical Medicine*, Am. ed., 1902, Philadelphia and London. Volume on Typhoid Fever.
Diendonné. *Centralbl. f. Bakt. u. Par.*, 1901, 30, p. 481.
Edel, P. *Fortschr. der Med.*, 1901, 19, p. 301.
Finkler, D. *Die acuten Lungenentzündungen als Infectionskrankenheit*, Wiesbaden, 1891, p. 216.
Flexner and Harris. *Johns Hopkins Bulletin*, 1897, 8, p. 259.
Foà and Bordoni-Uffreduzzi. *La Riforma Medica*, 1887, No. 1. (Abstracted in *Deutsche med. Wchnschr.*, 1887, 14.)
Fraenkel, A. *Deutsche med. Wchnschr.*, 1899, 25, p. 254.
Glaser, F. *Deutsche med. Wchnschr.*, 1902, 28, p. 773.
Gradwohl, R. B. H. *Interstate Med. Jour.*, 1899, 6, p. 329.
Jehle, L. *Wiener klin. Wchnschr.*, 1902, p. 232.
Karlinski, J. *Fortschr. der Med.*, 1889, 7, p. 681.
Lepine and Lyonnet. *Archiv. de med. exp.*, 1899, 11, p. 549.
Mallory, F. B. *Jour. Exp. Med.*, 1898, 3, p. 611.
Montier, P. *Thèse de Paris*, 1902.
Polguère, D. *Thèse de Paris*, 1888.
Rau, R. *Ztschr. f. Heilk.*, 1904, 25; *Abtheil f. Int. Med.*, p. 385. (Abstracted in *Amer. Med.*, 1904, 7.)
Roque and Bancel. *Lyon Médicale*, 1903, 100, p. 578.
Sahli. *Mittheilung aus klin. und med. Inst. der Schweiz*, 1894, 1, p. 749.
Stühlern. *Centralbl. f. Bakt. u. Par.*, 1900, 27, p. 353.

A THERMOSTABILE, HEMOLYTIC PRECIPITATE FROM NUTRIENT BROTH.

EDWIN O. JORDAN.

IN the ordinary technique of preparing nutrient broth the fresh beef infusion is heated, filtered to remove the coagulated albuminous matter, titrated and then brought to the phenolphthalein neutral point. The flocculent precipitate that is thrown down on neutralization is commonly assumed to be in part albuminous, and some writers even refer to it definitively as "acid albumin." The precipitate begins to form at about the phenolphthalein neutral point, and is deposited abundantly when the reaction is made —1.0 to —2.0.[1] Microscopic examination of the amorphous-looking precipitate shows it to be composed of a mass of fine, sharp, interlacing crystals. If the broth is made quite alkaline (e. g. —2.0 to —4.0), with either sodium, potassium or ammonium hydroxid, and the precipitate that at once forms immediately filtered off, large well-defined crystals develop in the broth on standing.

As a matter of fact the whole precipitate, after being washed with distilled water or 0.85 per cent NaCl solution does not give the biuret reaction or respond to any of the usual tests for proteid. So far as these tests indicate, the substance thrown down by the adding of alkali is entirely of a non-proteid nature.

The whole precipitate when thoroughly washed and suspended in 0.85 per cent NaCl solution is hemolytic for the corpuscles of the dog, rabbit and other animals.

When the reaction of the broth is made —2.0, the precipitate deposited has a slightly brownish tinge. If, however, the broth is first neutralized and the resultant precipitate removed, and the fluid is then made alkaline (—2.0) a second precipitate is formed composed of large white crystals. Both the whole precipitate (at the neutral point or at —2.0) and the purer precipitate after frac-

[1] Report of Committee on Standard Methods of Water Analysis, Supplement No. 1, *Jour. Infect. Dis.*, 1905, p. 106.

tional removal are hemolytic in substantially equal degree. Analyses show that the precipitates are almost identical in composition. The first precipitate, deposited at about the neutral point, owes its brown color to a small amount of iron salts; there is also a little calcium in the first deposit. The purer crystalline substance deposited in the slightly alkaline broth on standing has been subjected to thorough qualitative and quantitative analysis with the result that it has been found to be practically pure magnesium-ammonium-phosphate ($MgNH_4PO_4$). This substance is also the main ingredient of the deposit thrown down at the neutral point. Experiments with the chemically pure salt leave no doubt that the hemolytic property of the precipitate is due to the presence of the magnesium-ammonium-phosphate.

The salt is very slightly soluble in cold water or NaCl solution, and after long contact with it the supernatant fluid has practically no hemolytic effect. On heating some solution occurs.

If 0.05 gm. of this precipitate is evenly suspended in 5 c.c. of 0.85 of NaCl solution and this suspension is diluted to 1 to 100, it is found that 0.1 c.c. of this dilution will completely hemolyze the standard suspension of dog's corpuscles.[1] Chemically prepared magnesium-ammonium-phosphate gives the same results. Both the Mg and the PO_4 ion are devoid of hemolytic power and the inference is strong that the active factor in hemolysis by magnesium-ammonium-phosphate is the OH ion. In confirmation of this view may be mentioned the selective action upon dog corpuscles shown by the writer to be manifested by the hydroxyl ion.[2] A similar sensitiveness exists on the part of canine corpuscles towards the precipitate under consideration. The serum of a normal rabbit protects canine corpuscles against the action of alkali. Thus in one experiment 0.3 c.c. of normal rabbit serum protected canine corpuscles completely against the lytic dose of alkali and precisely the same amount of serum also protected against the lytic dose of the precipitate suspended in NaCl solution; in each case 0.2 c.c. of the serum failed to confer complete protection.

Many bacteria produce in broth cultures a degree of alkalinity quite sufficient to precipitate magnesium-ammonium-phosphate.

[1] *Jour. Med. Research*, 1903, N. S. 5, p. 33. [2] *Ibid.*, p. 31.

B. pyocyaneus, for example, in broth with an initial reaction of +1.0 will in a few days change the reaction to the alkaline side of the phenolphthalein neutral point and in a longer period will often make the reaction —3.0 to —4.0. The diphtheria bacillus can likewise alter the reaction of broth so that crystals of magnesium-ammonium-phosphate are formed.

These facts bear to some extent on the work with bacterial hemolysins. Some investigators who have worked with these substances state that the filtrate from bacterial cultures is less strongly hemolytic than the cultures themselves, and from this they have concluded that the hemolytic substance was "closely bound to the bacterial cell."[1] It is evident from the foregoing that the sediment in alkaline bacterial cultures contains a mineral substance capable in fine suspension of producing hemolysis.

[1] Bullock and Hunter, *Centralbl. f. Bakt.*, 1900, 28, p. 865.

A COMPARISON BETWEEN THE RESULTS OF BLOOD CULTURES TAKEN DURING LIFE AND AFTER DEATH.*

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So much has been done in recent years in the bacteriology of the blood and internal organs that we are all cognizant of the value of this method of diagnosis. In many cases the results of blood cultures are the only positive proofs of the nature of the infection, particularly in the large group of septicemic cases, where in the absence of some of the signs and reactions common to the better known infections such as the pulmonary signs, the sputum of pneumonia, the reaction of agglutination, and the skin pictures of many febrile diseases, the clinician is often much perplexed concerning the nature of the infection or intoxication at hand. Frequently at the present day the diagnosis can be made during life, and such a diagnosis made early may, with the future development of curative anti-sera, assume an importance equal to that of the recognition of diphtheria.

Much oftener, however, owing to a variety of reasons, these diagnoses are made postmortem, and the final decision rests with the pathologist or bacteriologist, who, however, has the clearness of his horizon to a greater or less degree clouded by the following facts: First, the occurrence of an agonal or pre-agonal migration of bacteria from the intestinal tract or lungs into the general circulation; secondly, the multiplication of organisms in the vessels and organs after death either already there, antemortem, or by invasion postmortem.

With regard to the latter it may be said that the wide-spread area of occupation in some instances suggests the likelihood of a deposition of bacteria in these parts before death, whence they

* Received for publication March 13, 1905.

wander freely postmortem, not by the arteries which are empty, but by the venous channels in which the blood remains fluid for some hours, or if coagulated, a sufficiency of serum still remains to facilitate progression.

Another factor to be considered in dealing with the presence of bacteria in the blood is the action of the bacteriolytic complement existing in the plasma. Longcope[1] believed that the presence or absence of terminal infections depended largely upon the amount of complement in the blood, a conclusion not wholly acceptable, since Weaver and Ruediger,[2] Rosenow,[3] Behring[4] and others have shown that upon streptococcus and pneumococcus normal sera exert no bacteriolytic action whatever. Nevertheless, as Longcope's experiments show, there does exist a certain definite antagonism against some bacteria, e. g., B. typhosus and B. coli, so that the activity of the complement content of the blood cannot be altogether discounted.

It seemed to us that a comparison of the bacteriological findings in the blood before and after death could not fail to be more than usually instructive, particularly since these results, if showing any similarity would have a doubly acting significance, the postmortem reading confirming the provisional antemortem bacteriological diagnosis. And the antemortem blood condition when in agreement with the postmortem would prove the value of this method of investigation and show to what extent contaminations, "agonal infections," and postmortem bacterial proliferation are to be considered in autopsy bacteriology.

Thanks to the courtesies of Professors Osler and Welch, we were enabled to make both ante- and postmortem bacteriological examinations of the blood in many cases, and to obtain positive results in a series of most interesting conditions. At autopsy the bacteriological investigations were conducted by one of us, while the blood cultures during life were taken by the other, using in both instances the utmost precautions necessary to reliable results. The technique of this operation during life, one which in innumerable cases has given us most satisfactory results, is briefly

[1] *Univ. Pennsylv. Med. Bull.*, 1902, 15, p. 331.

[2] *Medicine*, 1903, 9, p. 515. [3] *Jour. Infect. Dis.*, 1904, 1, p. 280. [4] Quoted by Rosenow.

as follows, the arm constricted tightly in the upper third, is scrubbed with green soap at the elbow fold, then alcohol is applied, either poured over, or rubbed over (the former by preference), succeeded by an application of ether in a similar fashion, followed by bichlorid of mercury 1:10,000–20,000, and lastly more ether or alcohol to dry off the skin. A large barrelled syringe (10 to 30 c.c.) was used and the needle inserted into one of the now prominent veins, several syringefuls of blood were frequently taken and either put into liquid media (broth, or milk and broth) or plated out in agar. These two methods of culture were employed, the first to diminish any bactericidal action by sufficiently diluting the blood, the second to avoid spoiling results by contamination, and to give an idea of the number of organisms present in the blood. At autopsy the cultures were preferably made from the heart's blood, the pericardium being burned with a red hot blade and opened with a lance, then the auricular heart muscle being similarly treated, a large platinum loop needle, or at times a small pipette, was introduced into the auricle and cultures made in agar plates, coverslips of the blood being usually taken at the same time, and cultures made from other organs.

The objections to the reliability of blood cultures, namely the possibility of taking contaminating organisms into consideration as infecting agents, requires a few words. When once a good technique had been established it was soon found that cultural results during life were in an overwhelmingly large per cent of cases quite sterile. In a few instances, not regularly, as one might imagine, knowing the difficulty experienced in sterilizing the skin, staphylococcus pyogenes albus in limited numbers, one or two colonies per plate, would appear in spite of all precautions. In direct proportion to lack of care in sterilizing apparatus, the ever present hay bacillus might be expected to overgrow the plates and tubes while other members of the contaminating horde might be met with. In a series of blood cultures embracing perhaps several hundreds of cases these two bacteria were practically the only contaminating organisms coming under our observation. It may be well to remark that we have never in our experience found unexpectedly or as contaminators in our cultures such prevalent

organisms as streptococcus pyogenes, B. coli, or staphylococcus pyogenes aureus, the plate method of culture having always to our mind been of great assistance in helping to rule out contaminations. Positive results give as a general rule several or many colonies per c.c. of blood in a plate; contaminating organisms, unless gross carelessness has been displayed, rarely number more than one or two colonies in a plate; in liquid media, of course, a contaminating microorganism may generally overgrow an infecting agent from the blood.

The ease with which positive results are now obtained in some of our well known clinically recognizable diseases such as pneumonia and typhoid fever, overrules most objections hitherto raised against the reliability of the procedure, and it is part of our task to show to what extent we may rely upon cultures to prove the existence of other less easily determinable infectious processes.

Many objections are urged against accepting unreservedly the results of postmortem bacteriology and many of these objections cannot be opposed. In from four to six hours after death some of the common organisms of the intestine are to be found in the neighboring organs unless the body has been carefully and immediately put in the refrigerator; this militates at once against many so-called colon-infections and infections supposedly due to similar and allied organisms. The presence of a single prevailing organism in exudates and inflammatory lesions may usually be accepted as evidence that the particular organism is the infecting agent, but infection of postmortem fluid transudates, in any of the serous cavities, is common and may occur early; the absence, however, of inflammatory reaction, in such cases, should prevent false interpretations of bacteriological results. We also recognize in routine work certain organisms as being more likely to be contaminating than infecting agents.

In cases of general infections, the subject with which we have to do, we are dealing with a fluid medium, the blood, which furnishes an avenue of connection between all parts of the body, and hence is open to all manner of infections, the sources of which, in many cases are apparent (local lesions) in many others are

obscure, as typhoid fever, pneumonia, rheumatism, epidemic cerebro-spinal meningitis (granting consideration to the general infection idea of these diseases). Postmortem invasion and infection of the blood from many sources is possible; postmortem proliferation of a few wandering organisms may give the bacteriological findings the appearance of a severe general infection; a postmortem contamination may obscure all bacteriological results. These are the general suppositions, it remains to be seen to what extent they are absolute, to what extent they are to be considered, practically, in other words, what credence may be given to reports of infections based on autopsy findings. As we have already stated, a correspondence between the blood cultures results before death and the postmortem cultural findings would cover all these points, and prove the value of the method of diagnosis.

Our table gives in detail the histories of the cases and the combined bacteriological reports.

Of fourteen cases considered there are seven in which the antemortem and postmortem results are absolutely the same; two in which the same organisms were present before and after death, but with the addition of another infecting or contaminating microbe in the postmortem cultures; five in which the antemortem results are not confirmed by the postmortem findings, or which show radical differences in cultural results.

In regard to the first group of cases, the seven showing absolute agreement in the antemortem and postmortem results, it need only be said that they are conclusive evidence of the value of blood culture and show that postmortem results may be accepted in a large per cent of cases. Closer inspection of the two next mentioned cases, Nos. 7 and 9, show that the postmortem difference is clearly due to an additional infection.

In No. 7 the deposition of streptococci and staphylococcus pyogenes aureus in the kidney and urine must be considered as happening in the so-called agonal period, their source from a local infection in the pleural exudate is apparent; cultures from the blood eight hours antemortem showed no sign of their presence, merely the heavy typhoid infection.

TABLE 1.

SHOWING CLINICAL, PATHOLOGICAL, AND BACTERIOLOGICAL DATA OF CASES.

Patient	Nature and History of Infections	Time of Cultures: Media Used	Antemortem Cultural Results	Anatomical Diagnosis and Postmortem Cultural Results
1. Jacob B. No. 24,628.	Cerebro-spinal meningitis. Fourth day of disease, onset with headache, vomiting and chill; retraction of neck, early; marked hemorrhagic rash on elbows and knees; nephritis (hematuria slight). On 5th day, general redness and swelling of joints; mottling of body surface; pneumonia right lower lobe. Temperature 101° on admission, rising to 105° at death. Unconscious and delirious.	Culture 20 hours A. M. *a*) Lumbar puncture: Loeffler's serum. *b*) Culture from knee joint; glycerine agar, agar plates. *c*) Culture from blood; glycerine agar plate, blood on surface; two agar plates; three plain blood undilnted.	*a*) 20 c.c. of turbid fluid: intracellular diplococci; growth of few typical colonies; *b*) 40 c.c. of stringy pus; numerous intracellular diplococci, growth of a few typical colonies; *c*) few typical colonies; growth in undilnted blood. Cultures and protocols indicate that from spinal fluid, knee-joint and blood, the meningococcus was isolated.	Meningococcus from meninges of cord and brain. Micrococcus lanceolatus from lung and blood of coronary artery.
2. A. S., 37 yrs.* Sept. 14, 1898. Oct. 3, 1898.	Rheumatism in 1889. Fever from July, 1898, to August; coming to hospital as supposed relapse in typhoid fever. Aortic valve disease present with fever; diagnosed malignant endocarditis and septicemia.	Culture in 9 days and 3 days A. M. from blood.	Both positive; micrococcus zymogenes.	Endocarditis of aortic valve. Septic infarcts in spleen and kidney. Micrococcus zymogenes in lung, spleen, kidney, gall bladder, aortic and mitral valves, and heart's blood.
3. Daniel S. 63 yrs. Carpenter. No. 21,513. Dec. 17, 1897. Dec. 20, 1897.	Pneumonia; onset 6 days previous with chill, pain in side, cough, consolidation in right side. Temperature 104° on admission, 101° at death. Gradual failure. Friction rub in right upper back. Heart irregular, no murmurs or pericardial friction. Leucocytes: on 17th, 32,000; on 20th, 20,000. Urine: albumen trace, few casts, numerous red blood cells, pus.	Culture on the 17th day A. M. *a*) agar; *b*) in Marmoreck's serum.	3 to 4 c.c. of blood. *a*) Numerous pin-point colonies; by protocols micrococcus lanceolatus. *b*) Contaminated. Mouse inoculated. ½ c.c. bouillion, culture fatal in 8 hours, diplococci in blood; micrococcus lanceolatus.	Micrococcus lanceolatus in heart's blood, lung, pleural exudate, and spleen.

* Reported by MACCALLUM AND HASTINGS, *Jour. Exp. Med.*, 1899, 4, 521.

TABLE 1—*Continued.*

Patient	Nature and History of Infections	Time of Cultures: Media Used	Antemortem Cultural Results	Anatomical Diagnosis and Postmortem Cultural Results
4. R.	Typhoid. History indefinite. Severe case, delirium, probably in 3d week. High fever. Widal positive. Death from intoxication, July 10, 1897.	Culture 4 to 5 hours A. M.	Abundant growth of typhoid bacilli.	Typhoid lesions. Typhoid bacilli in heart's blood, pericardial fluid, spleen, and mesenteric glands.
5. J. B.	Typhoid, severe case, 3d week: delirium, typhoid state, husky voice. Death from intoxication, Nov. 20, 1897.	Cultures on 8, 6, 4, and 1 days A. M.	8, 6, 4 days negative; 1 day colon bacilli.	Typhoid lesions and ulcers in larynx. Typhoid bacilli in liver, spleen, retroperitoneal and mesenteric glands, gall bladder, and urinary bladder. Streptococci in ulcers of larynx, esophagus, and heart's blood.
6. Kate W. Colored. 84 yrs. Servant. No. 25,221 Jan. 9, 1899. Feb. 1, 1899.	Arterio-sclerosis, chronic parenchymatous nephritis of several years, edema of feet and ankles, acute attack of headaches and weakness two weeks previous to admission, ascites and edema of legs. Systolic murmur at apex. No frictions. Temperature, irregular; 101.8 highest. Jan. 28th, development of pneumonia, diarrhea, vomiting. Delirium. Death on Feb. 1st. Leucocytes, 6,000. Temperature, Jan. 29th, 100°. " " 30th, } Normal. " " 31st, } " Feb. 1st, } Urine: albumen, hyaline and granular casts.	Culture Feb. 1st. *a*) Blood on agar plates. *b*) Blood in broth, 10 c.c.	*a*) Negative. *b*) Streptococcus pyogenes, 10 to 12 elements in chain. 2 c.c. of broth culture not fatal to guinea pigs.	Bacillus coli in kidney.

TABLE 1—*Continued.*

Patient	Nature and History of Infections	Time of Cultures: Media Used	Antemortem Cultural Results	Anatomical Diagnosis and Postmortem Cultural Results
7. Ryan. Mar. 25, 1899.	Continued case of typhoid; intermittent chills for 10 weeks. Thrombosis of right femoral vein, slight jaundice, developing coma. Friction rub at base of left lung. Urine: albumen, trace; bacilli present.	*a*) Cultures from urine repeatedly; *b*) from blood 6 to 8 days A. M. Bacilli in fresh blood.	*a*) Positive. *b*) Positive; bacillus typhosus.	Bacillus typhosus in blood, liver, bile, spleen, kidney, peritoneal fluid, urine and area of pleurisy. Streptococcus and Staphylococcus pyogenes aureus in kidney, pelvis, pleuritic adhesion, and in urine.
8. Jones. Oct. 19, 1898.	Arterio-sclerosis: endocarditis. Irregular temperature. Edema, dyspnœa.	Cultures 3 days A. M.	Positive; staphylococcus-pyogenes aureus.	Bacilli in heart's blood, urine, and peritoneal cavity. Staphylococcus aureus and albus in mitral valve vegetation.
9. P. O. Dec. 1, 1898. Feb. 1, 1899.	Lymphatic leukemia. Weakness and glandular swellings since 1896. Two previous admissions; improved and able to work. September, 1898, dyspnea and edema of legs, palpitation, diarrhea, cough: continuous arsenic treatment. Blood: R. B. C., 2,712,000; W. B. C., 44,000; hemoglobin, 40%. Leucocytes: small mononuclear, 89.5%; polymorphonuclear, 8.0%; eosinophiles, 2.0%; transitionals, 0.5%. Steady increase of leucocytes to 139,000. Pulse failed steadily; irregular temperature present on admission, more marked on Dec. 12, 1898—102.5 . Dyspnea, glandular swellings, cough all increased. Many infected papules on legs and hands. Died with no increase of symptoms on Feb. 1, 1899.	Jan. 18, 1899, 3 days A. M. Jan. 31, 1899, 1 day A. M.	Negative. Agar plates from blood, positive. Streptococci numerous.	Streptococcus in heart's blood, spleen, peritoneal cavity, inguinal glands, and edematous tissues in neighborhood. Mic. lanceolatus in lung.

TABLE 1—*Continued.*

Patient	Nature and History of Infections	Time of Cultures: Media Used	Antemortem Cultural Results	Anatomical Diagnosis and Postmortem Cultural Results
10. S. W. P. June 21, 1897. June 24, 1897.	Asthma: edema of legs. Acute rheumatism 30 years ago. Moderate drinker. Had jaundice 18 months ago; gastro-intestinal disturbances, 13 months ago; hematemesis, 10 days ago; edema, delirium, and jaundice marked; chest clear; some ascites, liver enlarged, spleen palpable. Albuminuria. Leucocytes, 20,000. Temperature, 101°–104°; 107° at death on June 24.	June 22, 2 days A. M. June 23, 1 day A. M.	Positive } Streptococci. Positive }	Hypertrophic cirrhosis. Streptococci in heart's blood, lung, bile, kidney, spleen.
11. J. A. 35 yrs. Colored. May 18, 1897. May 28, 1897.	Aortic insufficiency, edema, dilated heart, ascites, ulcer on leg. May 19, sudden rise of temperature to 104°, cough. Temperature remained elevated, reaching 106.5 on May 25. Died on May 28. No local signs to account for temperature. Urine albuminous, and a few blood casts.	Blood culture A. M.	Positive; streptococci.	Aortic insufficiency, endocarditis arterio-sclerosis. Streptococci from aortic valves, heart's blood, infarcts of spleen, and kidney.
12. F. 34 yrs.* Apr. 25, 1895. May 16, 1895.	Rheumatism for 3 months, weakness and dyspnea, severe chills, anemia, sweatings; physical signs of mitral stenosis. Leucocytosis. Urine: albumin and casts. Diagnosis: ulcerative endocarditis. Death May 16, 1895.	Cultures from blood, May 7, May 12, 1895.	Positive; mic. gonorrhoeae.	Ulcerative endocarditis of mitral valve: typical biscuit-shaped cocci decolorizable by Gram; cocci not able to be cultivated on media at hand.

*Reported by THAYER AND BLUMER, *Johns Hopk. Hosp. Bull.*, 1896, 7, 57.

TABLE 1—*Continued.*

Patient	Nature and History of Infections	Time of Cultures: Media Used	Antemortem Cultural Results	Anatomical Diagnosis and Postmortem Cultural Results
13. J. K. 19 yrs.* Feb. 5, 1896. Feb. 25, 1896.	Fever, weakness; gonorrhea 6 months ago; shaking chills Nov. 25, 1895; fever, sweats; progressive anemia and weakness; liver and spleen enlarged. Systolic murmur at base. Left knee swollen; urethal discharge, gonococci in same. Blood: R. B. C., 2,290,000; W. B. C., 9,000. Urine: albumen and pus. Left hospital Feb. 14, 1896; re-entered March 9, 1896, worse; urethal discharge less; friction over heart; petechiae on skin. Death March 25, 1896.	Mar. 22 Mar. 24. Mar. 25.	Negative. Positive } Mic. gonorrhoeae Positive }	Tricuspid endocarditis, hemorrhagic glomerulonephritis; acute sero-fibrinous pleurisy; pericarditis. Gonococci from pericardium, tricuspid valves, heart's blood.
14. Feb. 1, 1896.	History unknown. Pericarditis, pneumonia. Death Feb. 1, 1896.	Blood cultures a few hours antemortem.	Positive; streptococci.	A diplococcus from pericardial exudate and pneumonic area: micrococcus lanceolatus.

* Reported by THAYER AND LAZEAR, *Jour. Exp. Med.*, 1899, 4, 81.

TABLE 2.

SUMMARY OF CASES IN WHICH ANTEMORTEM AND POSTMORTEM CULTURES DIFFER.

Antemortem	Time of Culture	Postmortem
1.		
Meninges, knee joint, blood. Meningococcus.	20 hours A. M.	Meningococcus from cord, brain and meninges. Pneumococcus in blood and lungs.
5.		
Typhoid severe. Blood B. coli. No agglutination with B. coli.	4 cultures: 3 neg., 1 pos., 24 hours A. M.	B. typhosus in liver, spleen, retroperitoneal glands, mesenteric glands and gall-bladder. Streptococci in ulcers of larynx, oesophagus and heart's blood.
6.		
Arterio-sclerosis: temperature; pneumonia. Death afebrile. An aërobic streptococcus.	Few hours A. M.	Bacillus coli in kidney. Blood negative.
7.		
Typhoid fever. B. typhosus in blood, and seen in fresh blood: B. typhosus in urine.	6 to 8 hours A. M.	B. typhosus in blood, liver, bile, spleen, kidney, peritoneal cavity, urine and pleura; streptococcus and staphylococcus aureus in kidney-pelvis, pleura and urine.
8.		
Arterio-sclerosis; endocarditis; Staphyl. aureus.	3 days A. M.	Bac. in blood and peritoneal cav., Staph. aureus and albus on mitral valve.
9.		
Lymphatic anemia, chronic. Irregular temperature; broncho-pneumonia; streptococci in blood.	24 hours A. M.	Streptococcus in blood, spleen, peritoneal cavity, inguinal glands, and edematous tissues in neighborhood; mic. lanceolatus in lung.
14.		
Pericarditis; pneumonia. Blood cultures, streptococcus.	A few hours A. M.	Micrococcus lanceolatus from pericardial exudate, and pneumonic area in lung.

In No. 9 a suspected bronchopneumonia as evidenced by temperature and dyspnea probably allowed, and explains, the presence of the pneumococci in the blood and tissues, they not being present 24 hours antemortem.

In the last group of cases, five show considerable lack of correspondence between the ante- and postmortem cultures, and are more difficult of reconciliation.

No. 1, a case of epidemic cerebrospinal meningitis, the first reported instance of general infection with the meningococcus, the development of pneumonia explains the presence of the pneumococci in the blood postmortem. The non-development of meningococcus in the postmortem blood cultures may have been due to the feeble vitality of the organism, rendering cultivation difficult, or to their scarcity in or disappearance from the blood.

Case 5, a severe typhoid; four blood cultures failed to show the presence of the typhoid bacillus, in spite of the apparent

heavy infection found postmortem. The isolation of B. coli in this case 24 hours before death under such conditions is interesting and may be taken as an instance of a transitory bacteremia with its point of origin in some portion of the ulcerated intestinal tract; and since there was no serum reaction present in the blood for this bacillus, we must recognize that in this and similar cases infection of the blood stream may take place in the death agony, beclouding actual conditions and giving rise to varied postmortem results. Furthermore, a negative result, as is well known, may be met with several times in succession in this disease, so that the discrepancy is more seeming than real.

In Case 6, the lack of postmortem confirmation may have been due, on the one hand, to the disappearance of the organism from the circulation; on the other, to difficulty in cultivation or in not having taken a sufficiency of blood. The presence of B. coli may be held in the light of a postmortem migration.

The divergence of results in Case 8 meets with a possible explanation upon the grounds of a frank agonal or postmortem invasion; for the unidentified bacillus could scarcely have escaped recognition in the antemortem culture had it been present, considering its apparent widespread distribution. Staphylococcus aureus appearing in the mitral valve and not in the blood, might be accounted for by its being suppressed by the swarming in of the strange bacillus into the circulation or being crowded out in the plate. The presence of staphylococcus albus may possibly be due to an error in diagnosis, knowing as we do that some of the colonies amongst pigment producing bacteria at times fail to show pigment and their descendants for some generations may remain colorless.

The lack of harmony in Case 14 is undoubtedly caused by the development of pneumonia resulting in an admixture of organisms in the blood stream, with consequent difficulty of differentiation of the two types of colonies on the plate, the pneumonic infection being largely in the nature of an agonal one.

It will be seen, therefore, in such groups of divergent case results that no serious objections can be raised against the value of blood cultures during life. Failures or lack of harmony with

postmortem results may be caused, in the first place, by the non-appearance of organisms in the circulation in spite of the very definite clinical picture of an infection, due to causes at the present time imperfectly understood; in the second place, as was previously pointed out, to agonal or postmortem invasion by such organisms of the intestinal group as B. coli and its close relatives. In our experience infection during life with B. coli was a rare occurrence, and may be looked for where the gates are very widely open, as in conditions of extensive ulceration of the intestinal tract, or where there is lessened bactericidal action on the part of the blood toward such relatively feeble organisms as the members of the colon group.

Illustrative of a variety of conditions influencing the bacteriological results postmortem, we present a series of cases taken at random and shown in Table 3.

Without going into an exhaustive analysis of the cases, there are certain conditions worthy of remark. There is observable in Cases 1, 4, 5, and 9 a very definite polybacterial infection, due to doubtless to several factors; e. g., postmortem invasion, or multiplication of bacteria already there (5 and 9), to secondary local infection (1 and 4), or to the length of time existing between the death of the individual and the taking of cultures.

For unknown reasons, Cases 2, 6, and 8, all with surgical aspects, curiously enough do not supply us with as complex a picture as might be expected.

In cases illustrative of specific infective diseases, as typhoid fever and pneumonia — Cases 1, 4, 7, and 8 — the bacteriological findings confirm the anatomical diagnosis by the recovery of the specific microbes from the tissues. The failure to isolate Mic. lanceolatus where frank lobar pneumonia existed, in Cases 4 and 5, was caused, in all likelihood, by the accompanying polybacterial infection either totally suppressing growth or rendering it unobservable on the agar plates.

Any activity of the bactericidal power of the blood exhibited in these cases seems to be present in some degree in Cases 3 and 10, in the latter as long as 13½ hours after death.

It is evident from a perusal of the above table that with few

TABLE 3.

Number	Anatomic Diagnosis	Time after Death	Bacteriological Results
1....	Typhoid fever. Bronchopneumonia.	5 hrs.	B. typhosus: spleen, gall-bladder, mesenteric glands, and bone-marrow. Streptococcus Staphylococcus pyogenes aureus } Lung.
2....	Perforation of duodenal ulcer. General peritonitis.	5 hrs.	B. coli: heart's blood, spleen, gall-bladder, and peritoneal cavity. Streptococcus: peritoneal cavity.
3....	Perforation of duodenal ulcer. General peritonitis. Bronchopneumonia.	2 hrs.	Sterile: heart's blood. Mic. lanceolatus: lung.
4....	Typhoid fever. Lobar pneumonia.	31 hrs.	B. typhosus: gall-bladder and spleen. B. coli Streptococcus Staphylococcus pyogenes aureus } Lung.
5....	Lobar pneumonia. Bronchopneumonia. Abscess of parotid gland. Acute endocarditis.	12½ hrs.	Staphylococcus pyogenes albus B. lactis aërogenes } Lung. Bacillus (unidentified): lung, heart's blood, and parotid abscess.
6....	General peritonitis following colostomy.	12 hrs.	Streptococcus: spleen, liver, and kidney.
7....	Lobar pneumonia. Pericarditis.	8 hrs.	Mic. lanceolatus: heart's blood and lung. Sterile: spleen, and bronchial glands.
8....	Typhoid fever. Perforation.	20 hrs.	B. typhosus: spleen, liver, and gall-bladder. B. coli: liver.
9....	Hemorrhagic infarct of lung. Thrombosis of branch of pulmonary artery going to lower lobe. Acute bronchopneumonia.	13½ hrs.	Streptococcus: heart's blood and thrombus. B. pseudo-diphthericus: infarct. Staphylococcus pyogenes aureus B. coli } Thrombus.
10....	Chronic endocarditis.	13½ hrs.	Sterile: heart's blood.

exceptions uncontrolled postmortem findings must always be of more or less doubtful value, particularly if taken several hours after death and not associated with marked clinical symptoms. We must recognize, too, that almost all, if not all, the common bacteria of the intestines—particularly B. coli, B. lactis aërogenes, Streptococcus pyogenes, and B. pseudodiphthericus—may be met with in the circulatory channels and organs, probably as contaminating postmortem invaders, although one must admit that the mode of their entrance is not at all times clear. And it is here especially worthy of mention that the mere presence of these organisms in the blood should never be accepted as indisputable evidence of general infection, unless there exists confirmatory antemortem cultural findings, or definite clinical facts, such as

local lesions or the acknowledged signs of specific infectious diseases.

The presence of a variety of organisms on a plate at one time is always suggestive of contamination, unless there exists evidence of two distinct infective processes, as typhoid fever and pneumonia; but even such conditions as perforative peritonitis may show surprisingly paradoxical results on investigation of the inoculated media.

The presence or absence of the bactericidal power of the blood is, as Longcope has pointed out, probably responsible to a considerable degree for the great variation seen in a lengthy series of cases.

CONCLUSIONS.

As a result of our study of correlated ante- and postmortem cultures we offer the following conclusions:

1. That within certain time limits postmortem bacteriological methods afford trustworthy means of determining or confirming the presence of many of the well-known infectious processes.

2. That the more often a marked antemortem infection is present, the greater probability there is of finding an uncomplicated postmortem bacteriological result, provided the investigation is carried on within a reasonable time after death, say 12 hours.

3. That a bacteremia due to the common organisms of the intestinal tract and the so-called "agonal invasions" of the blood stream do occur, yet they should not be assumed to be present with any great degree of frequency.

4. That the rôle played by the bacteriolytic complements is probably considerable, although not entirely explaining in some cases the negative results during life with positive results after death, or *vice versa*.

5. That in view of the constant number of clinically unrecognized general infections in our hospitals, and the future possibilities of serum treatment, bacteriological investigation of the blood stream both before and after death should be more generally encouraged and practiced.

A CONTRIBUTION TO THE CLINICAL KNOWLEDGE OF TEXAS FEVER.*

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Texas fever, as is well known, is a malarial type of disease which attacks cattle in southern latitudes. It is characterized, as its name implies, by an elevation of body temperature. Hemoglobinuria commonly occurs.

The direct cause is a microparasite named (1889) from its appearance *Pyrosoma bigeminum* by Dr. Theobald Smith,[1] its discoverer.

It was shown in 1889–1890 by Kilborne[2] that a cattle tick of a particular variety (*Boophilis bovis*) is the natural transmitting agent.

These observers also showed that the disease may be produced at will, in a susceptible animal, by subcutaneously introducing blood from an "immune" animal, that is one that has apparently recovered from an attack of the disease.

Attempts to produce an antitoxic serum have proven unsuccessful.[3]

It has been observed that the disease produced in northern cattle by inoculation (injection of virulent blood) is less fatal than when it is acquired in the natural way, and that animals thus treated have a high degree of "immunity" after recovery.[4] Work along this line has largely been developed by Dr. J. W. Connaway of the Missouri Experiment Station and economic inoculation has been practiced by him with great success, the details of which may be found in the publications referred to. Dr. Conna-

* Received for publication February 4, 1905.

[1] Smith and Kilborne, *U. S. Bureau of Animal Industry, Bull.* 1893, 1, p. 177.

[2] *Loc. cit.*

[3] *Mo. Exp. Sta. Bull.*, 1897, 37; *Miss. Exp. Sta. Bull.*, 1898, 42; *ibid.*, 1899, 48.

[4] Smith and Kilborne, *loc. cit.*; J. W. Connaway, *Mo. Exp. Sta. Bull.* 37, 1897; Connaway and Francis, *Mo. Exp. Sta. Bull.*, 1899, 48, and *Texas Exp. Sta. Bull.*, 1899, 53; M. Francis, *Texas Exp. Sta. Bull.* 1902, 63; J. S. Hunt, *Report of the Inspector of Queensland Stock Institute*, 1897-8.

way published graphic records of the fever of seven bulls[1] and Dr. Francis a chart showing the course of the temperature and hematokrit readings of five heifers and one bull.[2]

Since these and other exact clinical observations hitherto made were not considered sufficiently exhaustive, I made a detailed study of the curve of temperature and the relative volume of corpuscles and plasma, as determined by the hematokrit, in 62 animals of different sex and various ages with a view of determining (*a*) the average curve of temperature and hematokrit readings, and (*b*) the influence of sex and age on these curves and the course of the fever in general. In addition to the temperature and hematokrit readings, in another animal, largely with the view of elucidating the remarkable hemolysis and hemoglobinuria already referred to, I investigated the chemical and morphological changes in the blood and physico-chemical changes in the blood and urine (Cow 2). I also made attempts to determine how long the blood retains its virulence when kept aseptically outside of the body at room temperature.

Chart 1 showing the average course of the temperature and hematokrit readings produced in cattle by inoculation is based on data gathered in eastern Kansas during July and August 1901 from a herd consisting of 62 head of registered Herefords. Of the cattle 41 were bulls and 21 heifers. They may be divided into four groups:

I. Thirty-five bulls ranging in age from 12 to 16 months;
II. Six bull calves, from about 6 weeks to 4 months;
III. Sixteen heifers, from about 12 to 16 months;
IV. Five heifer calves, from about 3 to 5 months.

On June 27 each animal of the herd received subcutaneously from 0.75 to 1.0 c.cm. of defibrinated blood obtained from an immune yearling steer. The mother of the steer was a small black Texas cow which was shipped to the Missouri Experiment Station August 1896. Before being shipped she had been dipped to free her from ticks and subsequently was several times inoculated from animals having the fever. Kept afterwards for two summers in a tick infested pasture, she gave evidence of no reaction. The sire of the steer was an immune registered Jersey. When a few weeks old, the steer was inoculated with blood from his mother and marked symptoms of fever appeared. Afterward he received blood from other animals immune to Texas fever. Ultimately he ceased to react to inoculation.

[1] *Mo. Exp. Sta. Bull.*, 1899, 48, p. 26. [2] *Loc. cit.*, p. 65.

The inoculated cattle were kept in stalls and fed a ration consisting of a mixture of ground oats, corn and wheat, with the exception of Groups II and IV, which in addition nursed. For roughness they were given new alfalfa hay. At night, owing to the heat and their need for water and exercise, they were turned into small lots adjoining the barn. During the day they were frequently sprayed with a preparation that kept from them the troublesome "horn flies."

The sick animals were sprayed with water to reduce their temperature and were given saline purgatives together with copious rectal injections of water to keep the bowels open. Temperature records were taken between six and eight o'clock in the morning and hematokrit readings from two to five in the afternoon.

In Chart 1 are displayed the curves of temperature and hematokrit readings determined by averaging results (1) of all the animals, (2) of the heifers, (3) of the bulls.

The inoculation fever may be divided into five periods, viz:

(1) *Primary incubation period*, from inoculation to the onset of fever (first to ninth day) is nine days long. During this time the percentage of sediment in the blood slightly decreases.

(2) *Primary fever period*, from the onset of the fever until the beginning of the intermediate period (9th to 18th day) is nine days long. The maximum temperature is reached on the sixth day, thus giving a relatively long *initial stage*. The *fastigium* is very short, being less than one day in length. The *stage of defervescence* is three days in length. Thus the fever terminates by *lysis*.

During the initial stage, the blood sediment sharply decreases. This is succeeded by a gradual increase during the latter part of the stage of defervescence.

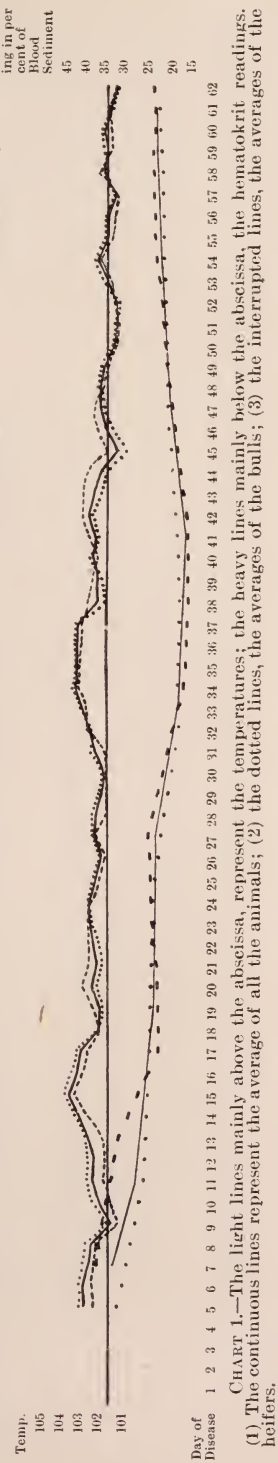

CHART 1.—The light lines mainly above the abscissa, represent the temperatures; the heavy lines mainly below the abscissa, the hematokrit readings. (1) The continuous lines represent the average of all the animals; (2) the dotted lines, the averages of the bulls; (3) the interrupted lines, the averages of the heifers.

(3) The term *secondary incubation period* may be appropriately applied to the interval from the end of the primary until the beginning of the secondary fever period (18th to 30th day). This is 12 days long. During this time the temperature is normal or slightly hypernormal. The blood sediment slowly increases during this period.

(4) *The secondary fever period*, from the end of the intermediate to the beginning of the convalescent period (30th to 38th day) is eight days long.

The *initial stage* is four days long. The maximum temperature for the period occurs on the last day of this stage and is slightly less than for the primary fever period. The *fastigium* is of three days duration. During this stage the morning temperatures remain practically constant. The stage of *defervescence* is only one day long, thus the fever terminates by *crisis*. During the initial stage, the blood sediment decreases more sharply and to a greater degree than in the corresponding stage of the primary fever. This decrease continues during the fastigium but during defervescence it begins again gradually to increase.

(5) *The convalescent period* may be divided into two stages:

(*a*) What may be called the *preconvalescent stage*, which lasts from the end of the secondary fever to the beginning of true convalescence (38th to about the 51st day) is about 13 days long. It is characterized by irregular hyper- and subnormal temperatures. The blood sediment continues to gradually increase.

(*b*) *The true convalescent stage*, which begins with the termination of the preconvalescent stage and terminates with the complete recovery of the animal. It may be roughly stated as being from 30 to 90 days in length. During this stage the temperature is normal and the blood sediment gradually returns to normal.

Charts of the individuals comprising the herd show that the course of the disease is often atypical as judged by the temperature and blood sediment records. In the case of the former the widest variations occurred, the curves showing in some cases practically no rise in temperature, while in others, the elevation was continuous throughout the time corresponding to the primary fever, second-

ary incubation and secondary fever periods. In some the primary elevation was absent; in others the secondary. When both elevations occurred, either might be the higher.

The curve of blood sediment was very constant as regards the primary and secondary decreases, both being present in every case. Variations as to time and amount of decrease are not uncommon.

The influence of age and sex on *the course* of (*a*) *temperature* and (*b*) *blood sediment* is shown in Table 1.

TABLE 1.

Groups Giving Sex and Ages	Maximum Primary Fever	Maximum Secondary Fever	Per Cent of Blood Sediment			
			Max. for Prim. Inc. Period	Min. for Prim. Fev. Period	Max. for Sec. Inc. Period	Min. for Sec. Fever Period
I. Bulls: Thirty-five young adults.....	104.9	104.8	31.0	23.4	25.2	12.4
II. Bulls: Six calves	104.2	105.0	35.0	28.1	29.6	12.3
III. Heifers: Sixteen young adults.....	104.8	104.7	37.3	23.0	27.5	14.0
IV. Heifers: Five calves.....	104.7	104.4	39.0	26.0	27.3	13.7

(*a*) Only in the group of bull calves does any considerable difference occur between the average maximum temperature of the primary and secondary fever periods. It appears that the primary fever was less in the calves than in the older bulls and heifers, and that of all the groups, the heifer calves had somewhat the lowest fever during both periods.

(*b*) During the *primary incubation period* the average maximum percentage of blood sediment was higher in both groups of heifers than in either of the groups of bulls. Also in both sexes it was higher in the calves than in the older animals.

In the *primary fever period* the lowest average minimum occurs in the older animals, being slightly less in the group of older heifers than in the older bulls. Also, in the calves, the average in the heifers was slightly lower than in the bulls. The percentage of decrease from the prior reading was also greater in both groups of heifers than in the bull groups. In both sexes, the decrease was greater in the older animals than in the calves.

During the *secondary incubation period* the bull calves showed the largest amount of blood sediment and the older bulls the smallest of any group. In the heifers it was slightly higher in the older animals than in the calves, and both groups were intermediate between the two bull groups. The percentage of increase was in both sexes greater, as might be expected from the previous decrease, in the older animals than in the calves, being much greater, however, in the heifers. In the calves, the increase was slightly greater in the bulls.

During the *secondary fever period* the minimum amount of blood sediment was lower and percentage of decrease from the previous readings greater in both groups of bulls than in either of the groups of heifers. In both sex groups, the minimium sediment in calves was slightly lower than in the older animals.

Influence of age and sex on nutrition and recovery is well marked. It is known that young animals stand the disease much better than old ones and that mortality is less among nursing calves than any other class.[1]

The calves, although showing high fever and low hematokrit readings, withstood the fever far better than the older animals. The bull calves rarely refused to nurse. The heifer calves also ate much more regularly than did the older animals. The loss of flesh was much less in the calves than in the older cattle.

That the disease was better withstood by the heifers in general is indicated by the following facts:

1) The mortality among the 41 bulls was 7.3 per cent; while none of the 21 heifers died.

From this it must not be inferred that the inoculation fever is never fatal to heifers. The mortality, however, is considerably lower than among bulls.

2) The disease did not have so great a tendency, in the heifers as in the bulls, to become chronic, i.e. irregular and protracted to beyond the usual time of termination.

3) Inactivity of the bowels and suppression of the urine, conditions often seen in the severer type of fever, was less frequent among the heifers than among the bulls.

[1] CONNAWAY, *Mo. Exp. Sta. Bull*, 1899, 48, p. 60.

4) That the nutrition of the heifers was less seriously impaired than that of the bulls, was shown by the former being less emaciated, their recovery more rapid and by their eating more regularly during the disease.

The period at which death is most liable to occur, according to these observations, is during the secondary fever period, from the 36th to 40th days. But, as Dr. Connaway[1] has stated it may occur during the primary fever period. There was also one death in my series during the convalescent period. The following protocols give data bearing on this point.

Three of the oldest bulls died of the disease viz., No. 6 on the 16th, No. 71 on the 36th and No. 69 on the 37th day of inoculation, Bull No. 6 died at a time corresponding to the stage of defervescence of the primary fever. The fever began on the seventh and rose irregularly to 106.4 on the 13th day. The fastigium lasted two days, or until the 15th during which time the maximum temperature was 106.6. The fever terminated by crisis, a sharp fall in the temperature from 106.2 to 102.3 occurring the day prior to death. On the fifth day of inoculation, the hematokrit showed 29.0 per cent of sediment; on the 11th, which was the fifth day of the initial stage, 25.0 per cent. From this time onward until the 15th day, when the reading was 10.0 per cent, the decrease was sharp and fairly constant from day to day. The 16th, the day death occurred, the reading remained at 10.0 per cent. This would indicate that death was due to some cause other than diminution of the blood sediment, particularly as in several other similar animals, inoculated at the same time, recovery occurred after the hematokrit had shown only seven per cent of sediment.

Bull 71 died on the second day of the stage of defervescence of the secondary fever period. The temperature curve is unusual in this case. The temperature during the incubation period was variable. The primary fever period began on the 13th and terminated on the 18th day. The inital stage was two days in length, during which time the temperature rose gradually from 101.6 to 103.6. During the secondary incubation period, the temperature was normal, ranging from 102.0 to 102.6 until the last day, when it sank to 101.0. The secondary fever period began on the 30th day. The initial stage terminated on the 35th during which time the temperature irregularly rose to 104.8. From this point it gradually fell for two days until it was 101.5 which was the day of the death of the animal.

The blood sediment showed no decrease up to the 15th, the day of maximum primary fever, when it was 30.0 per cent. On the 22d, it showed a decrease to 26.0 per cent. On the 29th, which was the day preceding the secondary fever, it was 25.0 per cent. The next and last reading, which was on the 36th, was 10.0 per cent.

This shows that the primary loss of sediment was very slight and even more greatly delayed than the primary elevation of temperature.

[1] *Mo. Exp. Sta. Bull.*, 1899, 48, p. 58.

Bull 69 exhibited temperature and blood sediment curves very like those of No. 71; therefore a detailed description is unnecessary. The only essential difference occurs in the temperature curve of the secondary fever, which began on the 28th day. The temperature rose fairly regularly to 104.5 on the 32d. It remained practically constant until the 35th, when it was 104.0, giving a fastigium three days long. The fever terminated by crisis, falling to 100.6 on the next, the 36th and last day. The blood sediment was 26.5 per cent on the 29th and 10.0 per cent on the 36th.

In addition to these three animals, Cow No. 2 (temperature and hematokrit records are given in Table 3 and Chart 2), died from inoculation *, death occurring, after well marked primary and secondary periods, on the 40th day, corresponding to the fifth day of the preconvalescent stage.

Of 63 head of cattle of different sex and ages, four deaths occurred from inoculation as follows: one at the end of the primary fever period; two at the end of the secondary fever period and one during the beginning of the preconvalescent stage. These figures would indicate that the period of greatest mortality is between the 36th and 40th day after inoculation.

Through the courtesy of the Missouri Experiment Station, the cows used in the following experiments were placed at my disposal during the summer of 1903.

Cow 1, the immune animal used for supplying the blood for inoculation, was the Texan described on p. 530. She was in good condition, being intermediate between the other two.

Cow 2, used for inoculation, was a well bred Jersey about twelve years old. Her condition was medium and she was in the third month of pregnancy.

Cow 3, used as a control, was a registered Jersey belonging to the dairy herd. She was eight years old, in excellent condition and in the third month of pregnancy.

From the data obtained, Table 2, showing the *individual* and *average* normals, was prepared.

All the cows were kept in a good blue-grass pasture which had an abundance of shade and running water. Throughout the period of the experiment, they had access to salt and hay.

Blood for microscopical examination was obtained from the three animals on the afternoon of July 13, by puncturing the marginal ear vein. On the afternoon of the 14th about 1000 c.cm. of blood was aseptically drawn from each by inserting a trochar into the external jugular vein and about half of this

* In considering the mortality resulting from inoculation in these experiments from an economic standpoint it should be borne in mind that aged cows like Cow 2 almost invariably die.

TABLE 2.

Number of Cow	Hematokrit per cent of Sediment	Number of Red Corpuscles per cu. mm.	Volume Index of Red Corpuscles	Number White Corpuscles per cu. mm.	Proportion of White to Red Corpuscles	Hemoglobin Estimation (Tallquist)	Hemoglobin Estimation (Dare)
1.....	37.0	6,124,000	0.596	5,520	1:1109	95.0	50.0
2.....	36.0	5,591 000	0.648	4,700	1:1174	97.5	64.5
3.....	45.0	6,205,000	0.725	3,400	1:1823	100.0	60.0
Average	39.3	5,773,000	0.656	4,560	1:1368	97.5	58.1

Number of Cow	Hemoglobin Estimation (Sp. Gr. Method)	Color Index (Hb. Estimation by Tallquist)	Color Index (Hb. Estimation by Dare)	Blood Sedime't Hemoglobin Ratio	Sp. Gr. of Blood	Sp. Gr. of Serum	Sp. Gr. of Blood Sedime't	Osmotic Resistance of the Red Corpuscles
1.....	85.0	0 778	0.409	1.28	1.0570	1.0330	1.096	0.54
2.....	74.0	0.878	0.581	1.35	1.0545	1.0350	1.088	0.54
3.....	95.0	0.806	0.483	1.11	1.0600	1.0300	1.096	0.54
Average	84.6	0.820	0.491	1.24	1.0571	1.0326	1.093	0.54

Number of Cow	Coagulation time of Blood in Seconds	Fibrin of Blood	Alkalinity of Blood	Alkalinity of Serum	Chlorides of Serum	Diastatic Ferment of Blood	Diastatic Ferment of Serum	Coagulable Proteid of Serum
1.....	190	0.704	133.2	77.3	0.500	0.024	0.025	....
2.....	168	0.692	98.6	95.9	0.504	0.017	0.018	4.95
3.....	168	0.350	103.9	79.9	0.546	0.023	0.021	....
Average	175.3	0.581	111.9	84.4	0.516	0.021	0.022	....

Number of Cow	Δ of Blood	Δ of Serum	Relative Electrical Conductivity of Blood	Relative Electrical Conductivity of Serum	Sp. Gr. Urine	Alkalinity of Urine	Chlorides of Urine	Urea of Urine	Δ of Urine
1.....	0.549	0.549	127.2	112.5	.....	....	....	.....	.....
2.....	0.578	0.569	100.0	100.0	1.031	94.0	0.55	0.019	1.947
3.....	0.581	0.555	136.3	112.5	1.022	55.0	0.76	0.011	.....
Average	0.569	0.558	.....	.....	.....	....	....	.....	.....

was, in each case, defribrinated and the rest allowed to clot. The various portions of blood were sealed in sterile glass jars which were kept on ice until the examinations and analyses were finished

The hematokrit readings were made independently by two individuals, the average of the two readings being recorded. In no instance did the difference in the readings amount to more than 0.25 as read on the scale of the tube. Daland's hematokrit was employed which gave a speed of 10,000 revolutions per minute.

The number of red corpuscles was determined in the blood obtained before coagulation from the ear and in defibrinated blood drawn from the jugular vein. The differences, if any, are negligible. The Thoma-Zeiss apparatus with the Türck ruling was employed. Nine-tenths per cent NaCl was used for diluting the blood. It is considered superior to more concentrated solutions often recommended for this purpose, owing to the fact that I found the counting easier when the corpuscles were uncrenated. Possible error due

to the more rapid settling of the corpuscles that occurred, than when stronger solutions are employed, was avoided as far as possible by mounting rapidly. The greatest care was exercised in determining the exact dilution and in making perfect mounts. A block of 36 small squares was counted in each corner of the ruled field and another as near the center as possible. This gave the total for 180 squares. Another mount was then made and the count repeated, the result of which was averaged with the first, and the total number per cu. mm. calculated. The variation between the two counts was always very small. In a few instances the counts were identical.

The volume index of the red corpuscles was determined by the Capps' method,[1] the formula used being the per cent normal hematokrit reading divided by the per cent normal number, and expressing the result decimally.*

The number of white corpuscles was determined by means of the same instrument used in the red count, only the appropriate pipette was used. Three per cent acetic acid was used for laking the red corpuscles and diluting. The same care was exercised as in the case of the red count and the same method used excepting 2304 small squares were counted each of the two counts, giving a total of 4608.

The percentage of hemoglobin was determined by two methods: Tallquist's and Dare's. Readings were taken by three individuals, the recorded number being always an average of the three readings. In general, the individual variations were much less with the Tallquist than with the Dare method; also the readings by the former method agree more closely with the amount of hemaglobin indicated by the hematokrit, red count, and specific gravity of the blood.[2] But neither method is sufficiently accurate to give really reliable information as regards absolute amount of hemoglobin.

The color index was calculated in the usual manner, i. e. by dividing the percentage normal number of red corpuscles into the percentage of normal hemoglobin and expressing the result decimally. The results are recorded in two columns in the table, one based on the hemoglobin estimation by Tallquist's and the other by Dare's method. The results are considered to be of little actual value, the main reasons for including them in the tables being for completeness and reference. They are not sufficiently consistent in Cow 2, (Table 3) to render it possible to draw any general conclusions from them, doubtless because the average size of the corpuscles was no longer normal. To meet this difficulty, another factor called *the blood sediment Hb ratio*, obtained by dividing the percentage normal hemoglobin (Tallquist's method) by the percentage normal total volume of sediment as determined by the hematokrit, is used. By reference to Table 3, it may be observed that this factor more nearly expresses the condition of the blood coloring matter, judging mainly from the hemoglobin estimations, specific gravities and hemotokrit readings, than do the color indices.

The specific gravity of the blood was determined by Hammerschlag's method the samples consisting of defibrinated blood from the jugular.

* In calculating the volume and color indices, the normal hematokrit reading was taken as 50; the number of red corpuscles as 5,000,000 and the hemoglobin as 100.

[1] CAPPS, *Jour. Amer. Med. Assn.*, Feb. 16, 1901, 34, p. 464.

[2] Hammerschlag's method. See EWING, *Clin. Diag.*, 2nd ed., 1903, p. 57, 58.

The specific gravity of the serum, in addition to the above method was determined by a hydrometer, the results of the two methods being practically identical.

The specific gravity of the blood sediment, the term applied to the factor used to indicate the richness of the blood sediment in hemoglobin, was calculated by the following formula :

$$\text{Sp. Gr of Sediment} = \frac{(100 \times \text{Sp. Gr. of blood}) - (\text{percentage of serum} \times \text{Sp. Gr. of the serum})}{\text{percentage of sediment}}$$

Where absolute rather than comparative results are desired, of course the hematokrit readings of sediment and serum must be corrected for the serum which remains in the interstices of the former, even after prolonged centrifugalization. Ordinarily this would certainly not amount to more than 10 per cent of serum for Eykman found only about 10 per cent of serum in the blood sediment centrifugalized at a speed of 2,600 per minute for one and one-half hours.

Stewart[1] found in 100 c.cm. of blood sediment after a second prolonged centrifugalization at about the same speed only 9.31 c.cm. of serum. Hamburger[2] points out that the amount of serum left in the sediment is proportional inversely to the speed of the hematokrit; therefore the sediment obtained with a speed of 10,000 revolutions per minute of the hematokrit contains in my experiments probably less than this amount of serum. In abnormal conditions the amount may vary, particularly any condition tending to alter the specific gravity of the sediment relative to the specific gravity of the plasma or serum or the character of the sediment, would be calculated to cause a variation. Without great accuracy in the hematokrit readings the factor is of little value as of course a small variation in the percentage of sediment would give a relatively large variation in the result.

In determining the *osmotic resistance of the red corpuscles* the method of Hamburger[3] was used. The readings were made at the end of one hour at room temperature. The figures given represent the minimum strength NaCl solution in which the corpuscles retained their hemoglobin.

The coagulation time was determined by inserting a trochar into the jugular vein, collecting the first 5 to 10 c.cm. of blood in a test tube and observing the time of beginning coagulation.

The amount of fibrin was determined by drawing and measuring a quantity of blood and then collecting the fibrin by whipping and straining through gauze. The fibrin was then washed in water until it became white after which it was preserved in three per cent formaldehyde. About a month after the last was obtained, the several amounts were removed from the formaldehyde and thoroughly washed in water and then extracted with alcohol-ether, after which it was dried at 110° C. and then weighed. In the table the amoumts represent the weight of dry fibrin in gms. per 100 c.cm. of blood.

[1] STEWART, *Jour. Physiol.*, 1899, 24, p. 371.

[2] *Osmotischer Druckund Ionenlekre*, 1902, 1, p. 515.

[3] *Ibid.*, p. 61.

The alkalinity of the blood and serum was determined essentially by Engel's modification of Loewy's method.[1] One c.cm. of defibrinated blood being mixed with 20 c.cm. of distilled water and the mixture titrated with $\frac{n}{75}$ tartaric acid, the point of *slight* acidification being determined by testing with neutral litmus paper. In the case of the serum the titration was also performed after adding to the diluted serum a few drops of neutral litmus solution. The results by the two methods were the same. The blood and serum used were taken from the jars filled from the jugular, the test being made an hour after drawing the blood, excepting on the day of inoculation, owing to an unavoidable delay, 36 hours elapsed before the titration could be made. Therefore, the alkalinity given as normal in Table 2 is probably too low. The figures represent mg ms. NaOH per 100 c.cm.

The chlorides of the serum and urine were estimated by Salkowski's modification of Volhard's method[2] and expressed as gms. NaCl per 100 c.cm.

The diastatic ferment of the blood and serum was estimated as follows: To 25 c.cm. of a boiled starch solution in a test tube, made by adding two grams sugar free starch to 200 c.cm. of distilled water and boiling and then making up to 250 c.cm., one c.cm. of defibrinated blood or serum was added being deposited near the surface of the solution to insure thorough mixing. The sterile cotton plug was then replaced and the tube left at 25° C. for 48 hours at the end of which time its reducing power was estimated by Fehling's solution. The figures in the table represent the amount of reducing sugar in gms. found in 25 c.cm. of the starch solution. The tubes were set up within two hours of the time the blood was drawn.

The coagulable proteids of the serum and urine were determined quantitatively by the method of Roberts and Stolnikow as modified by Brandberg, which in numerous experiments in the laboratory has been found to give surprisingly accurate results. The figures are in terms of percentage.

Freezing point determinations were made by Beckman's method.[3] The average of three readings was recorded. In no case was the individual variation more than 0.005°. Usually it was less than 0.003°.

Relative electrical conductivity measurements were made by Kohlrausch's method[4] and the results are stated in the tables as percentage of conductivities on the day of inoculation, the measurements of the blood and serum on that day and of the urine on the third day being stated as 100 per cent in each case.

The urine was collected in the afternoon, the amount secured ranging from a few hundred to several thousand c.cm. No attempt was made to collect it for 24 hours at a time as it was impracticable under the circumstances.

The specific gravity of the urine was determined by means of an urinometer.

Urea was estimated in the urine by the sodium hypobromite method, the amount being expressed in terms of gms. per c.cm.

[1] *Berl. klin. Wchnschr.*, 1898, 35, p. 308.

[2] *Ztschr. f. physiol. Chem.* 1882, 5, p. 285.

[3] *Ztschr. phys. Chem.*, 1888, 2, p. 638.

[4] KOHLRAUSCH AND HOLBORN, *Das Leitvesmögen und Elektrolyte*, 1898.

TABLE 3.

Day of Inocula'n	Temp. 8 A. M.	Hematokrit per cent of sediment	Number of red corpusel's per cu. mm.	Volume index of red corpuscles	Number of white corpuscles per cu. mm.	Proportion of white to red corpuscles	Hemoglobin estimation (Tallquist)	Hemoglobin estimation (Dare)	Hemoglobin estimation (Sp. Gr. method)	Color index (Hb. estim. by Tallquist)	Color index (Hb. estim. by Dare)	Blood sediment Hb. ratio	Specific gravity of blood	Specific gravity of serum	Specific gravity of blood sediment	Osmotic resistance of red corpuscles	Coagulation time of blood in seconds	Fibrin of blood
−1		36.0	5,591,000	0.648	5040	1 : 1173	97.50	80.00	70.0	0.878	0.58	1.35	1.0545	1.0350	1.088	0.54*	168	0.692
0			5,451,000	0.660	4760	1 : 1602	97.50	64.50		0.894	0.69	1.35						
3	101.1	36.0	5,451,000	0.660	3400	1 : 1602	97.50	73.00		0.974	0.50	1.50						
6	100.4	32.0	4,940,000	0.647			96.25	50.00		1.183	0.61	1.57						
10	101.4	31.0	4,120,000	0.752	2772	1 : 1487	97.50	52.00		0.925	0.64	1.49						
13	100.3	25.5	4,120,000	0.618	2177	1 : 1872	76.25	54.20		0.962	0.51	1.48						
16	102.0	20.0	3,500,000	0.571	1526	1 : 2337	67.50	38.25	53.0	0.700	0.70	1.62	1.0475	1.0335	1.098	0.52	315	0.359
17	103.0	21.5	5,109,000	0.430	1464	1 : 3407	70.00	46.00		0.916		1.47						
23	101.0	24.5	3,959,000	0.619	3611	1 : 1099	72.50			1.153	0.43	1.07						
36	98.9	11.0	1,025,000	1.073			23.75	9.00		0.960	0.31	1.22						
37	99.0	9.5	1,212,000	0.780	2000	1 : 606	23.25	7.50	32.0				1.0380	1.0350	1.066	0.48	350	0.802

Day of inoculation	Alkalinity of blood	Alkalinity of serum	Chlorides of serum	Diastatic ferment of blood	Diastatic ferment of serum	Coagulable protein of serum	Δ of blood	Δ of serum	Relative electrical conductivity of blood	Relative electrical conductivity of serum	Specific gravity of urine	Alkalinity of urine	Chlorides of urine	Urea of urine	Coagulable protein in urine	Δ of urine	Relative electrical conductivity of urine
0	98.6	95.9	0.504	0.017	0.018	4.95	0.569	0.578	100.0	100.0	1.032	94.4	0.55	0.030	none	1.947	100.0
3											1.024	384.0	0.40	0.028	none		
13	255.8	197.2	0.614	0.007	0.008		0.574	0.577	118.1	100.0	1.023	472.0	0.40	0.020	none	1.462	48.4
17											1.016	328.0	0.20	0.018	none		
19	223.9	169.5	0.614	0.012	0.013		0.671	0.684	136.3	118.7							
37											1.010	400.0	0.05	0.018	0.165	0.866	16.2
38											1.027	164.0	0.07	0.013	0.330		
40			0.710			1.98											

The first line (− 1) represents the day before, the second the day of, and the third the day after inoculation.
* On the 8th day it was the same.

TABLE 3—*Continued.*

Day of inoculation.....	3	4	5	6	7	8	9	10	11	12	13	14	15
Temp. 8 a. m...	101.1	100.7	100.4	100.4	100.0	100.1	101.0	101.4	101.0	100.9	100.3	101.0	102.3
Hematokrit readings.....	36.0		35.5	32.0		32.0		31.0		26.0	25.5		25.0
Day of inoculation.....	16	17	18	19	20	21	22	23	24	25	26	27	28
Temp. 8 a. m...	102.0	103.0	102.5	103.0	102.3	101.3	101.0	101.0	101.3	100.7	100.7	101.4	103.0
Hematokrit readings.....	20.0	21.5	21.0		21.0	24.0		24.5				20.5	
Day of inoculation.....	29	30	31	32	33	34	35	36	37	38	39	40	
Temp. 8 a. m...	103.2	105.0	105.2	104.0	102.4	101.7	98.3	98.9	99.0	100.8	100.7	99.2	
Hematokrit readings.....	18.0	17.0	14.5	14.0	12.0	9.0	10.5	11.0	9.5	9.0	9.0		

The alkalinity of the urine was determined by titration with $\frac{n}{10}$ oxalic acid, using blue litmus solution for the indicator. The results are expressed in terms of mg ms. NaOH per 100 c.cm.

On Cow 2, after inoculation with 25 c.cm. of defibrinated blood immediately after it was drawn from the immune Cow 1, temperature readings (rectal) were taken daily at 8 A. M. Hematokrit tests were made at 4 P. M. The results are displayed in Chart 2 and Table 3.

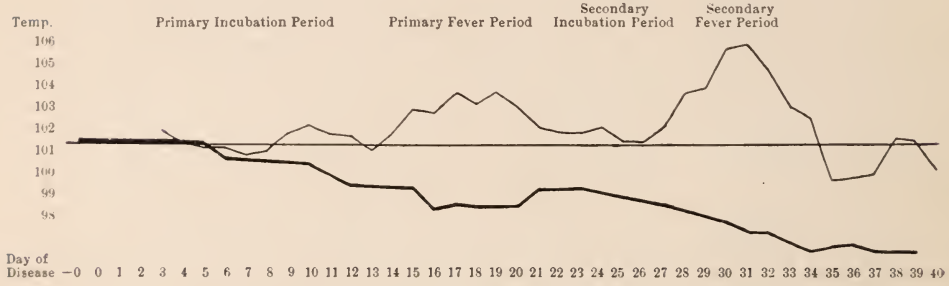

CHART 2.—The light continuous line represents the temperature; the heavy continuous line, the hematokrit readings.

The blood used for microscopical examination, etc., was obtained from the ear vein, excepting on the day of inoculation, and the 17th and 37th, when it was drawn from the jugular and defibrinated.

The serum was obtained from jugular blood on the day of inoculation, the 17th and 37th, and from blood from the heart at postmortem, which was within two hours of the death of the animal, on the 40th.

The urine was collected as described above excepting that in one instance a catheter was used and that on the 40th day it was taken from the bladder at the postmortem.

THE COURSE OF THE FEVER AS SHOWN BY THE CLINICAL FINDINGS.

THE TEMPERATURE READINGS.

The incubation period, 13 days in length, was normal for eight days. The first temperature was taken on the third day and it is slightly hypernormal, probably owing to the excitement of the animal which was unused to the manipulation. Subsequently readings on different mornings averaged about 100.5° and varied from each other by 0.0° to 0.4° until the eighth, when a period of hypernormal temperature began, which terminated on the 13th.

Between the 8th and 10th the temperature rose to 1.3° from which point it gradually decreased until the 13th, when the decrease amounted to 1.1°.

The primary fever was late, beginning on the 13th and lasting to the 22d, or nine days. The temperature increase from the 13th to the 15th was regular and amounted to 2.0°. On the 16th, a decrease of 0.5° was observed. On the 17th an increase of 1.0°, which was the maximum (103°) for the period.

The fastigium lasted from the 17th to the 19th. The fever during this time was of the remittent type. On the 18th, the temperature showed a decrease of 0.5°. The 19th showed a return to 103.0.

The stage of defervescence was from the 19th to the 22d. The first day the temperature decreased by 0.7°; the second, 1.0° and the third, 0.3°.

The secondary incubation period was four days in length, extending from the 22d to the 26th. Until the 24th, the temperature was practically constant. On the 25th,a drop of 0.4° occurred. The last day no change was observed.

The secondary fever period was about eight and one-half days in length, extending from the 26th to between the 34th and 35th. The *initial stage* was five days long, the increase in temperature being fairly regular until the maximum (105.2) was reached, which was 2.2° higher than in the primary fever. The *fastigium*, if present at all, was less than one day in length.

The period of defervescence was about three days long, extending from between the 31st and 32d to between the 34th

and 35th. The temperature decreased with fair regularity to below normal on the last day.

The period of convalescence was characterized by a sharp drop in temperature to subnormal during the first day. The next three days it rose to about normal, from which point it gradually decreased until death occurred, which was between the fifth and sixth days of this period.

The diurnal variations: At five o'clock on the afternoons of the 29th, 30th, 31st, 32d, and 35th days after inoculation, the temperature was 106.3°, 106.0°, 106.0°, 106.2° and 100.9° respectively. The morning temperatures for the same days were 103.2°, 105.0°, 105.2°, 104.0°, and 98.3°. This shows an irregular diurnal variation of 1.0° to 3.1°. In healthy cows of the same age the diurnal variation is only about 1° to 2°.[1] Accordingly the term *continued remittent* might appropriately be used to describe the fever.

THE BLOOD.

The blood sediment normally amounted to 36.0 per cent. On the third day after inoculation no change was observed. A marked decrease was seen on the sixth, from which time until the 10th the decrease was very slight and gradual.

The observation made on the 12th showed a sharp decline. From this date until the 15th, the decrease was very slight. On the 16th, another sharp drop occurred and the minimum for the primary fever period was reached (20.0 per cent). From this point until the 20th on the whole a slight increase occurred. The 21st showed a marked increase which continued more gradually until the 23d, the day on which the maximum was observed for the secondary incubation period (24.5 per cent). From this date was observed a regular marked decrease until the 34th, at which time the minimum for the secondary fever period (nine per cent) was reached. Until the 36th, a slight increase occurred, succeeded by a fall which continued until the 38th, when the amount of sediment was the same as on the 34th. No further change was observed.

The number of red corpuscles decreased until the 10th day coincidently with, and was proportional to the decrease in the blood sediment as evidenced by the volume indices.

[1] CONNAWAY, *Mo. Exp. Sta. Bull.*, 1897, 37, p. 106.

The decrease shown on the 10th day in the number is greater than the hematokrit reading would indicate. Of course when the average volume of the corpuscles is altered as in the disease under discussion the hematokrit reading ceases to be a reliable criterion of the number.

For instance, if we take the normal number for this individual as 5,500,000 and the normal hematokrit reading as 36.0 per cent then one per cent of sediment represents 150,000 corpuscles. On the 10th day, the hematokrit reading (31.0 per cent) indicated $31.0 \times 150{,}000 = 4{,}650{,}000$ corpuscles, while the actual count was 4,120,000.

The white count on this date showed a decrease also; therefore the increase in the total volume could only have been brought about by an increase in the individual size of the red corpuscles, or by the presence of suspended matter in the blood other than intact formed elements. Careful microscopical examination yields no evidence to support the latter hypothesis. A general increase in the size of the corpuscles is therefore the most probable explanation, as the microscope did not show any megalocytosis at this time.

Three days later, the 13th day of the disease, and next to the last day of the incubation period, the number of red cells remained unchanged, but the hematokrit showed a decrease to 25.5 per cent. A large increase in the number of the white cells occurred during this period, but this alone will not account for all the loss in sediment. The volume index shows that the average size of the red cells is slightly less than normal.

On the 16th day of inoculation the count was still greater, relative to the hematokrit reading, although the white cells had decreased almost 50 per cent since the last observation. The red cells must therefore have continued to decrease in volume.

On the 17th day, the number of the reds had increased greatly and the hematokrit reading slightly, the whites remaining practically unchanged. The volume index sharply declined to 0.43, the minimum observed.

The number of corpuscles and volume index on the 23d were again the same as at the beginning of the primary fever period. This corresponds to the middle portion of the secondary incubation

period. The next count made on the 36th day, corresponding to the beginning of the preconvalescent stage, showed the lowest number (1,026,000) and largest volume index (1.075) observed.

The relatively large volume index is explained in part, at any rate, by the morphological changes seen at this time (megalocytosis and relative increase in the number of the larger varieties of white corpuscles).

On the 37th, the day the last count was made, there was a slight increase in the number of red corpuscles and a decrease in the volume index. This corresponds to the last elevation of temperature. The morphological findings were practically the same as the day before.

On the whole then it would seem that from after the onset of the fever to its termination, the volume index of the red corpuscles is sub-normal. Table 4 and the following protocols, giving data obtained during 1901, substantiate this conclusion.

TABLE 4.

Day of Inoculation	Temperature 7 A. M.	Per Cent of Blood Sediment	Number Red Corpuscles per cu. mm.	Volume Index of Red Corpuscles
6	102.5	32.0		
to				
15	106.6	10.0		
15	106.6	10.0		
to				
18	102.5	11.0		
19	102.7	11.0	3,015,000	0.37
20	105.5	15.0		
21	105.5	16.0	3,105,000	0.50
22	104.6	14.0	2,461,000	0.51
23	103.1	15.0		
24	102.9			
25	103.1	15.0	2,402,000	0.62
26	102.8	16.0	3,508,000	0.43
27	105.8	17.0	3,762,000	0.40
28	103.7	17.5		
29	102.3	18.0		
to				
39	100.6	25.0		

Bull 4, Group 1.

Heifer No. 21 (Group 3) showed a primary fever period from the 17th to 25th day, the temperature rising to 105.0 on the 19th. On the 20th it was the same, thus giving a fastigium two days in length. The first day of defervescence, the temperature was 103.5; the count 6,960,000 and the blood sediment 30.0 per cent, giving a volume index of 0.43.

Heifer No. 15 (Group 3) had a very short primary fever lasting from the 19th to 23d day, The secondary fever began on the 32d day. The maximum

temperature during the primary fever (104.3) occurred on the 21st day. The blood sediment increased from 25.0 on the 20th to 40.0 per cent on the 24th. The number of red corpuscles on the latter date was 7,575,000 giving a volume index of 0.52.

The increase in the volume index which occurred on the 10th can only be due to an increase in the average size of the corpuscles. This swelling might be occasioned by a decrease in the concentration of the blood plasma. Unfortunately, no freezing point determinations were made on this date. Changes in the permeability of the envelope of the corpuscles might account for the increase in volume, even though the osmotic pressure of the plasma remained unchanged. It is conceivable that such a change in the envelopes could result mechanically from the presence of the causative organism or chemically from products of its metabolism; or from specific lytic substances produced by the tissues of the host as the result of the presence of the organism or its products; or to the action of other substances, such as bile salts, present in the blood in sufficient quantity to exert a hemolytic action, the first stage of which, in general, is swelling.

The sharp increase in the number and the decrease in volume at the beginning of the fastigium is most probably due to a change in the total volume of the plasma, or a reversal of the changes in the envelope indicated above, and not to any change in the molecular concentration of the plasma, as the freezing point was practically unaltered. With the exception of a decrease in the number of free nuclei, shown by a count with a high power (14,400 per cu. mm. as against 20,000 on the preceding day), no morphological changes occurred. As there were many microcytes present and a high power was not used in counting the corpuscles it is not improbable that some of the free nuclei may have been mistaken for corpuscles. For this reason the red counts may be somewhat inaccurate. The apparent decrease in the volume index is probably partly explained in this way. The animal died three days later without any more counts being made.

The decrease in the number of white corpuscles began slightly prior to the beginning of the decrease in the number of the red corpuscles and in the blood sediment, that is between the first and third days. The decrease on the third day amounted to about 28 per

cent of the total number. This decrease continues until the 17th which corresponds to the onset of the fastigium of the primary fever period. A decrease occurred between the 16th and 17th days. It will be remembered that on this day a sharp increase occurred in the number of the red corpuscles. The next count made on the 23d, corresponding to the intermediate period, showed an increase in the number existing between the first and third days. The next and last count, made on the 37th day showed a further decrease.

The proportion of the white to the red corpuscles decreased until the 17th day (3.1:1173 to 1:3407). The next count on the 23d showed a return to almost normal proportions (1:1099). The proportion on the 37th day was hypernormal (1:606). Thus it appears that the actual number of white corpuscles is decreased throughout the incubation and primary fever period; that it approaches normal during the intermediate stage and that during the secondary period it probably again decreases as the number taken on the 37th day shows a marked decrease from that of the intermediate period.

The percentage of hemoglobin begins to decrease between the third and sixth day. The decrease is gradual, but marked until the 16th day. A slight increase occurs on the 17th day corresponding to the onset of the fastigium of the primary fever period. A sharp increase was found on the 23d, which corresponds to the intermediate period. Just after the secondary fever period (37th day) a lower percentage of hemoglobin (about 20.0 per cent of normal) was found than was observed at any other stage. In all probability the percentage was still lower just prior to the death of the animal.

Most clinical observers agree that the Tallquist method gives a reading somewhat higher than the amount of hemoglobin actually present. The reading by this method averaged about 28° higher on the scale than by the method of Dare. The estimation based on the specific gravity of the blood showed a maximum increase in the hemoglobin amounting to 50 per cent.

In the blood sediment Hb ratio, we get a general increase to the 16th day and then a slight decrease to the 23d day, but still

it was somewhat above the normal. On the 36th day, the amount of hemoglobin per unit of sediment was considerably below normal. This agrees with the microscopical appearances and the general conclusions drawn from the variatious in the color index in similar conditions of the blood in man.

The specific gravity of the blood was markedly decreased on the 17th and still more on the 37th day. The specific gravity of the serum was slightly less than normal on the 17th. On the 40th day the serum had normal specific gravity. Judging from the quantitative analysis on the 37th and 40th, the specific gravity on the 37th could not have been far from normal. The specific gravity of the serum therefore varies but slightly during the disease.

The osmotic resistance of the red corpuscles slightly increased, the beginning of which was between the 8th and 17th day. A further slight increase was seen in blood taken from the heart at postmortem.

The coagulation time of the blood which on the day of inoculation was 168 seconds was markedly increased on the 17th to 315 seconds, and on the 37th it was still further increased to 350 seconds.

The amount of fibrin was much less than normal on the 17th, but on the 37th it was more than normal.

Obviously, then, the coagulation time is not proportional to the amount of fibrin. The most plausible explanation of the increase in coagulation time is that the fibrin ferment was slow in forming owing to the decrease in the actual number of, and abnormal proportion of kinds, of white corpuscles. Also the greater concentration of the plasma and the presence of bile salts may have played a part.

The amount of coagulable proteids was determined quantitatively in serum from blood drawn on the day of inoculation and in serum from blood taken at the postmortem. A marked decrease was seen on the 40th day, a decrease so great that it could not in any case be explained as due to inaccuracies of the method used in estimating.

The alkalinity of the defibrinated blood was slightly higher than of the serum, on the day of inoculation, the 17th and the 37th days.

The alkalinity of each was less on the 37th than on the 17th.

The chlorides of the serum were increased during the disease, being about 20 per cent higher on the 37th day and about 40 per cent higher on the 40th, than on the day of inoculation.

It is unfortunate that the determination was overlooked on the 17th day.

The diastatic ferment of the blood is decreased during the disease. The amount present on the 17th was less than on the 37th day. The serum contained more ferment per c.cm. than the defibrinated blood.

Freezing point observations on the blood and serum were the same on the 17th as on the day of inoculation. On the 37th, the Δ of the serum was increased by 0.107° and that of the blood by 0.097°. From this it is evident that a marked concentration of the plasma occurred between the 17th and 37th days. All observations showed a slightly greater Δ for the serum than for the defibrinated blood. On the 17th, the difference was only 0.003°, which is quite within the error of observation. But on the 37th the difference was 0.013° and on the day of inoculation 0.009°, while the differences found in successive observations on one and the same serum or blood never exceeded, as has been previously stated, 0.005° and were usually not more than 0.003°.

The electrical conductivity of the blood showed an increase on the 17th day, due doubtless to decrease in the total volume of corpuscles. The conductivity of the serum was unchanged. On the 37th day there was a further increase of the conductivity of the blood. This, of course, was also largely due to diminution in the total volume of the corpuscles. But it must also be attributed in part to the marked increase in the conductivity of the serum, which is associated with the increase in chlorides (and possibly other inorganic salts). The decrease in coagulable proteids also must of course cause an increase in the conductivity of the serum, since non-electrolytes depress the conductivity in proportion to their concentration.

Bile salts were demonstrable in the serum from blood taken on the 37th day by the following method:

Twenty-five c.cm. of serum were added to 75 c.cm. of 95 per cent alcohol and carefully sealed to prevent evaporation. Some

months later, the mixture was filtered and the filtrate tested by evaporating two to three c.cm. in a porcelain dish and testing the residue by Pettenkofer's method. The result was positive, a very faint purple stain being obtained. The alcoholic filtrate gave no visible rotation to the plane of polarized light when tested in a polariscope.

THE URINE.

The specific gravity gradually decreased from 1.032 on the third to 1.009 on the 37th day owing to a decrease mainly in the percentage of inorganic solids. On the 40th day it was 1.027.

The alkalinity increased to five times its original amount between the third and 17th days. A marked decrease was observed on the 19th day and on the 38th an increase again but not to the same degree as on the 17th day. On the 40th it was again markedly decreased.

The chlorides decreased irregularly from the third to the 19th day. The first decrease was observed on the 13th. No further change occurred to the 17th day. Two days later (the 19th) an additional decrease was found. On the 38th the amount was practically the same as on the third day. An increase to above the original amount was found on the 40th day.

The Urea showed an increase the third and 13th days. On the 19th and 38th days it was decreased slightly, the amounts on the two days being practically the same. The 40th day showed a further decrease.

Coagulable proteids were not demonstrable on the 3d, 13th, 17th or 19th days. Amounts equivalent to 0.165 and 0.330 per cents were found on the 38th and 40th days respectively.

The Δ (*depression of freezing point*) was 0.485° less on the 17th and 1.081° less the 38th than on the third day. Since electrical conductivity was diminished this was probably largely due in both instances to a decrease in electrolytes.

The relative electrical conductivity showed a very marked decrease on the 17th and a still greater decrease on the 38th as compared with the third day.

The diminution in the chlorides explains a portion of this decrease but is not sufficient to account for it all. The presence

of albumin on the 38th would act in the same direction but since a gram of albumin in 100 c.cm. of urine would only diminish the conductivity by about two per cent, a considerable portion of diminution of conductivity must be due to a decrease in electrolytes other than chlorides.

Up to the 40th day, no blood pigment was observed in the urine. On this day, when examined in a thick layer by transmitted light, a reddish color was seen. Spectroscopic examination showed a single faint band corresponding in position to that of reduced hemoglobin.

The morphology of the blood in Cow 2 was studied in fresh blood before drying and in film preparations made by spreading the blood thinly on cover-glasses, drying in the air, fixing by heat, staining with alcoholic eosin and alkaline methylene blue, drying and mounting in Canada balsam.

The following notes were made:

On day of inoculation.—Red cells of good color and well formed. A few are larger than the average, but the latter predominate. The white cells are mainly basophile, consisting largely of lymphocytes and eosinophile leucocytes.

Third day.—Red cells unchanged. White cells appear to show slight increase in eosinophile leucocytes.

Sixth day.—Red cells unchanged. White cells show relative predominance of lymphocytes.

Tenth day.—Red cells show no obvious change. White cells in many cases do not appear normal. Of five eosinophile leucocytes observed, four were in various stages of disintegration. They appear to be more numerous than on the sixth. Also a few groups of free nuclei, evidently once belonging to polynuclear white cells were seen.

Thirteenth day.—Red cells unchanged. White cells apparently show a small relative increase in the polynuclear basophile leucocytes.

Sixteenth day.—Red cells not obviously changed. The white cells show marked changes. Many of the polynuclear leucocytes of the slightly basophile variety showing a kind of unipolar staining with eosin, were seen. Also cells of the larger polynuclear variety with the protoplasm distinctly and evenly stained with eosin were observed. These abnormal types together with the eosinophile leucocytes represent from 40 to 60 per cent of the total number of the white cells, the remainder being mainly lymphocytes.

Seventeenth day.—The red cells apparently show fewer megalocytes than on the 16th; a considerable number of microcytes were present. White cells very few in number. Lymphocytes appear to predominate.

Twenty-first day.—Red cells appear same as before. Possibly fewer microcytes. White cells, no change observed.

Twenty-third day.—Red cells same as on 21st. No nucleated reds observed up to this time. White cells appear same as on 21st.

Thirty-sixth day.—Red cells show marked poikilocytosis. About 20 per cent are nucleated and many free nuclei are present. The cells stain poorly with eosin. White cells more numerous in proportion to the reds. Apparently the lymphocytes predominate. Many eosinophile and basophile polynuclear varieties are present.

Thirty-seventh day.—Red cells same as last time or poikilocytosis is more marked. White cells appear same as before.

The postmortem was made within two hours of the death of the animal. The findings were characteristic of the disease.[1]

Specimens from all the visceral organs were preserved and subsequently embedded, sectioned, stained and mounted, but space forbids a presentation of the results here.

A fetus about four months old was removed from the uterus. Blood obtained by cutting the umbilical vessels was subcutaneously injected into an animal susceptible to the fever. The result was negative. Amniotic fluid was secured and on examination gave the following results:

Color, clear light yellow.

Sp. Gr., 1.009.

Alkalinity, 28.0 mg ms. NaOH per 100 c.cm.

Urea, 0.0027 gms. per c.cm.

Chlorides, 0.68 gms. per 100 c.cm.

Coagulable proteids, negative.

Reducing power for Fehlings solution equivalent to 0.0006 gms. dextrose per c.cm.

Spectroscopic examination, negative.

With a view of determining if isolysins or autolysins were produced by the disease, the following experiments were made:

On the day of inoculation, the sera from the three cows were carefully examined for isolysins. In order to be sure of getting a reaction if any active substance was present, tubes containing equal parts of serum and five per cent suspension of washed corpuscles in nine-tenths NaCl were employed. To save space the results are not given in detail. The following conclusions were arrived at:

1. Serum of Cow 1 was slightly hemolytic for the washed corpuscles of Cows 2 and 3.

2. Serum of 2 was slightly hemolytic for the washed corpuscles of 3, but not for 1.

3. Serum of 3 was markedly hemolytic for the washed corpuscles of 2, but not for 1.

Control tubes of serum and corpuscles from one and the same animal gave negative results in each case.

[1] CONNAWAY, *Mo. Exp. Sta. Bul.*, 1897, 37, p. 90.

The time of "spontaneous" or "auto" laking was determined by putting five c.cm. of sterile defibrinated blood from each animal on the day of inoculation into sterile test tubes which were plugged with sterile cotton and kept in an amber glass bottle at 25° C. No laking occurred until the third day, when the blood from Cows 2 and 3 showed slight laking, the latter a little more than the former. At this time that from Cow 1 was unchanged. Of course the corpuscles settled to the bottom of the tube and laking was readily detected by observing the clear serum at the zone of contact with the sediment.

On the fourth day, the blood of Cow 1 was still unlaked.

On the eighth day, the tubes from cows 1 and 2 showed moderate laking while that from Cow 3 was strongly laked. Microscopical examination showed no bacteria and there was no putrifactive odor.

On the 17th day after inoculation blood from Cow 2 was tested again in the same manner. No laking was visible four days later, the 21st day. Again on the 37th day, the test was made on Cow 2. Three days later, the 40th, a very slight amount of laking was visible on close inspection.

Blood obtained from the heart at postmortem by aseptic technique and tested in the same way, was not laked 24 hours later.

From this it may be safely concluded that the time of "spontaneous" laking was not greatly altered in Cow 2.

It is hoped that an opportunity may be had to extend these observations on animals having the severer symptoms of the disease.

It has been stated that blood from an immune cow kept aseptically for several days will produce the disease on inoculation into a susceptible animal, and that such aseptic blood shows extra-corpuscular forms of the specific plasmodium. I therefore inoculated defibrinated blood from Cow 1 (the immune) kept aseptically as 25° C. for 14 days into a susceptible animal but obtained a negative result notwithstanding the fact that, microscopically, this blood after incubation showed numerous bodies corresponding to the descriptions and plates of the extra-corpuscular forms above mentioned.

Further investigations along this line are yet necessary and will be prosecuted at the first opportunity.

In conclusion, I wish to acknowledge indebtedness to certain members of the medical faculty of the University of Missouri for their furtherance of this research, particularly to Dr. Connaway. Also I am indebted to Dr. G. N. Stewart for help in the revision of the manuscript.

following the last passage through serum, as nearly uniform emulsions as possible were made of each serum-grown culture and of its control. To three c.c. of each emulsion were added three c.c. of normal rabbit serum, the two thoroughly mixed, $\frac{1}{10}$ c.c. immediately removed and plated to determine the initial bacterial content, and the remainder then placed in the thermostat. Subsequent platings at the intervals indicated in the table (Table 1) were made in the same manner.

TABLE 1.

Serum Resistance after 10 Generations in Active Serum.

Hours	B. prodigiosus		B. proteus vulgaris		B. fluorescens	
	Serum Culture	Agar Culture	Serum Culture	Agar Culture	Serum Culture	Agar Culture
0	43,600	30,200	53,800	61,200	33,500	40,000
1	24,200	22,700	45,400	40,500	30,100	32,500
3	36,300	28,000	80,000	54,300	9,500	28,000
6	50,000	47,000	120,000	110,000	10,200	21,000
12	110,000	97,000	225,000	262,500	46,000	35,200

The test for serum resistance shows that no one of the cultures has undergone any change as the result of the action of the serum. The serum-grown cultures possibly overcame the action of the serum slightly more rapidly, but the difference is so small that little importance can be attached to it.

One fact which attracts attention is the strong natural resistance of these organisms to the bactericidal action of the serum. According to Buxton,[1] one c.c. of normal rabbit serum may be expected to kill 1,000,000 typhoid bacilli, 50,000,000 paratyphoid bacilli, and 100,000,000 cholera vibrios. By experiment I determined that one c.c. of normal rabbit serum would kill only 300,000 proteus vulgaris bacilli, and 350,000 prodigiosus bacilli. This serum was tested on B. typhosus and found to have the usual bactericidal action on this organism. More evidence of the low bactericidal action upon these nonpathogenic organisms was shown by the fact that even during the first passages growth invariably occurred in the highest dilutions where comparatively few bacilli were subjected to the action of the serum.

[1] *Jour. Med. Res.*, 1905, 13, p. 305.

The first animal inoculation of this series was made 10 days after the last passage through serum. Four c.c. of a 24 hour broth culture of B. prodigiosus was injected into the peritoneal cavity of a full-grown rabbit. At the end of 10 hours the animal was found dead. Cultures were made from the peritoneal cavity and heart, and the organism isolated in pure culture. Further animal inoculations with this series were now made (Table 2). All inoculations were of 24 hour broth cultures and were made intraperitoneally.

TABLE 2.

ANIMAL INOCULATIONS AFTER 10 GENERATIONS IN NORMAL SERUM.

CULTURE		AMT. OF 24 HOUR BROTH CULTURE	ANIMAL AND WEIGHT		RESULT
			Rabbits	Guinea-Pigs	
B. prodigiosus	Control	8 c c.	1,730 gms.	...	Slightly sick; recovered
	Control	6	1,370	...	No effect
	Serum culture	4	1,345	...	Dead in 10 hours
	Serum culture	2	1,550	...	Very sick; recovered
	Control	4	.....	365 gms.	No effect
	Serum culture	4	.....	354	" "
B. proteus vulgaris	Control	6 c.c.	1,545 gms.	...	No effect
	Serum culture	4	1,640	...	Dead in 20 hours
	Serum culture	2	1,720	...	" " 14 days
	Control	4	.....	360 gms.	No effect
	Serum culture	4	.....	370	" "
B. fluorescens	Control	8 c.c.	1,640 gms.	...	No effect
	Serum culture	4	1,620	...	Dead in 12 hours
	Serum culture	2	1,700	...	Sick; recovered
	Control	4	.....	415 gms.	No effect
	Serum culture	4	.....	422	" "

There was a very marked increase in the virulence of each organism for rabbits, but no increase for guinea-pigs was detected.

Second active serum series.—In order to test further the relation between serum resistance and virulence, the series through active serum was repeated. This second series consisted of a total of 20 passages and extended over a period of about 12 weeks.

After the first three passages through the serum, the cultures were tested to determine if, at this stage, there was any evidence of serum resistance. It was found that each serum-grown culture was slightly less resistant to the action of the serum than was its

control. Evidence of this fact was also seen in the slant cultures taken from the plates following each passage, after a larger number of passages the slant cultures being noticeably more vigorous than at first. The power of pigment production by B. prodigiosus was greatly enhanced. The serum culture would produce an amount of pigment in 18 hours which it would take the control culture 48 hours to equal.

After the last of the 20 passages the cultures were transferred daily on agar for three days before making the resistance and virulence tests.

Table 3 gives the serum resistance after 20 generations in active serum.

TABLE 3.

SERUM RESISTANCE AFTER 20 GENERATIONS IN ACTIVE SERUM.

HOURS	B. PRODIGIOSUS		B. PROTEUS VULGARIS		B. FLUORESCENS	
	Serum Culture	Agar Culture	Serum Culture	Agar Culture	Serum Culture	Agar Culture
0	5,200	3,000	9,000	4,300	3,300	10,000
1	600	600	4,500	2,800	3,000	8,000
3	950	400	8,000	8,600	900	7,500
6	1,500	1,600	30,000	40,000	1,000	5,000
12	30,000	28,000	60,000	65,000	10,000	8,000

In this series after 20 generations, as in the first series after 10 generations, the serum-grown cultures have developed no appreciable resistance to the bactericidal action of the serum. The results from this standpoint are entirely negative.

As before, both rabbits and guinea-pigs were inoculated (Table 4). All inoculations were made intraperitoneally with 24 hour broth cultures.

For rabbits the M. L. D. of B. prodigiosus had been decreased from near eight c.c. to about one c.c.; of B. proteus vulgaris, from over six c.c. to two c.c.; and for B. fluorescens there was probably a slight increase in virulence, although this was not definitely determined.

For guinea-pigs, the M. L. D. of B. proteus vulgaris alone had decreased, three c.c. being fatal, whereas four c.c. of the control was not fatal. More accurate determination was not made.

TABLE 4.

ANIMAL INOCULATIONS AFTER 20 GENERATIONS IN ACTIVE SERUM.

CULTURE		AMT. OF 24 HOUR BROTH CULTURE	ANIMAL AND WEIGHT		RESULT
			Rabbits	Guinea-Pigs	
B. prodigiosus	Control	8 c.c.	1,730 gms.	...	Slightly sick; recovered
	Control	6	2,405	...	No effect
	Serum culture	2	1,250	...	Dead in 4 hours
	Serum culture	2	1,185	...	" " 3 "
	Serum culture	1½	1,175	...	" " 48 "
	Serum culture	1	1,540	...	" " 14 days*
	Control	4	.....	365 gms.	No effect
	Serum culture	4	.....	335	" "
	Serum culture	4	.....	392	" "
B. proteus vulgaris	Control	6 c.c.	1,920 gms.	...	No effect
	Serum culture	4	2,110	...	Dead in 12 hours
	Serum culture	4	1,650	...	" " 12 "
	Serum culture	2	1,360	...	" " 16 days*
	Control	4	.....	360 gms.	No effect
	Serum culture	4	.....	285	Dead in 12 hours
	Serum culture	4	.....	340	" " 12 "
	Serum culture	3	.....	310	" " 12 "
B. fluorescens	Control	8 c.c.	1,640 gms.	...	No effect
	Serum culture	4	1,700	...	Slightly sick; recovered
	Control	4	.....	415 gms.	No effect
	Serum culture	4	.....	300	" "

*Organism isolated in all cases except those marked with asterisk.

Inactive serum series.—For comparison with the above results, a series of passages (20 generations) were made through rabbit serum inactivated by heating at 55–56° C. for one-half hour.

B. fluorescens was not tested for serum resistance, but the other two organisms show no change as a result of the passages (Table 5).

TABLE 5.

SERUM RESISTANCE AFTER 20 GENERATIONS IN INACTIVE SERUM.

HOURS	B. PRODIGIOSUS		B. PROTEUS VULGARIS	
	Serum Culture	Agar Culture	Serum Culture	Agar Culture
0	4,800	5,200	5,940	5,000
1	800	1,500	1,320	600
3	1,025	1,200	2,640	2,100
6	7,400	3,700	11,000	6,400
12	43,000	36,000	74,000	5,400

The animal inoculations with this series gave the following results.

TABLE 6.

ANIMAL INOCULATIONS AFTER 20 GENERATIONS IN INACTIVE SERUM.

CULTURE		AMT. OF 24 HOUR BROTH CULTURE	ANIMAL AND WEIGHT		RESULTS
			Rabbits	Guinea-pigs	
B. prodigiosus	Control.....	8 c.c.	1,730 gms.	...	Slightly sick; recovered
	Control.....	6	2,405	...	No effect
	Serum culture..	4	1,490	...	" "
	Serum culture..	4	.....	365 gms.	" "
B. proteus vulgaris	Control.....	6 c.c.	1,920 gms.	...	No effect
	Serum culture..	4	1,425	...	Very sick; recovered
	Serum culture..	4	1,400	...	Dead in 12 hours
	Serum culture..	4	1,375	...	" " 4 "
	Serum culture..	2	1,060	...	" " 4 "
	Control.....	4	.....	360 gms.	No effect
	Serum culture..	4	.....	335	Dead in 12 hours
	Serum culture..	4	.....	315	" " 12 "
B. fluorescens	Control.....	8 c.c.	1,640 gms.	...	No effect
	Serum culture..	4	1,430	...	Dead in 12 hours
	Serum culture..	2½	1,440	...	" " 14 days
	Control.....	4	.....	415 gms.	No effect
	Serum culture..	4	.....	305	Dead in 12 hours

There is here no indication of an increase in virulence of the inactive serum culture of B. prodigiosus for either rabbits or guinea-pigs. The virulence of B. proteus vulgaris, however, is very much increased for rabbits and is somewhat increased for guinea-pigs, the exact degree for the latter not being determined. B. fluorescens also shows an increased virulence for both rabbits and guinea-pigs.

SUMMARY OF RESULTS.

1. None of the three slightly pathogenic or "nonpathogenic" organisms (B. prodigiosus, B. proteus vulgaris, and B. fluorescens nonliquefaciens) showed any change in serum resistance as a result of passage through either active or inactive normal rabbit serum.

2. Upon passage through active rabbit serum all three organisms increased in virulence for rabbits, B. prodigiosus at least sixfold. B. proteus vulgaris and B. fluorescens did not give quite so high increases in virulence. B. proteus vulgaris and B. fluorescens increased in virulence for guinea-pigs, but B. prodigiosus did not.

3. Upon passage through inactive serum, B. prodigiosus showed no increase in virulence for either rabbits or guinea-

pigs. B. proteus vulgaris showed an increase for rabbits equal to that obtained when passed through active serum. B. fluorescens showed a greater increase in virulence for guinea-pigs than upon passage through active serum.

In conclusion I wish to express my gratitude to Dr. E. O. Jordan for his interest and valuable suggestions during the course of the work.

THE BACTERIOLOGICAL EXAMINATION OF A PLAGUE RAT, WITH NOTES ON THE CAPSULAR SUBSTANCE FORMED ON NUTRIENT AGAR BY SOME BACTERIA.*†

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On March 15, 1905, a rat found dead in the house of Dr. Lugay, 106 Calle Principe, was sent to the Laboratory by Dr. W. P. Read, Station A, San Nicholas, Manila. I had never felt satisfied that plague occurred in rats in Manila, so the cultures isolated were subjected to a careful study.

The routine examination of a large number of rats becomes so onerous that most laboratories adopt some rough and ready methods of diagnosis. In Manila the diagnoses were based on microscopic examinations. In Japan and China (Hong Kong) similar methods have been relied upon. In India less questionable methods are in vogue, the formation of involution forms on salt agar being relied upon in Central India (Hankin), while in the Plague Research Laboratories at Parel, Bombay, the formation of stalactites in flasks of broth is the criterion.

While the use of one, or better both, of the last two methods is all that can be expected when a large number of rats must be examined, it would certainly seem advisable to supplement this work by a few more carefully conducted bacteriologic examinations, especially when plague first appears in a new locus, or when only a few rats are found dying of a plaguelike disease.‡

An acquaintance with the work of Dunbar and Kister,[1] and especially with that of Neumann,[2] on the occurrence of plaguelike microorganisms in rats will emphasize the point I wish to make. One grows tired of reading of the occurrence of plague in pigs,

* Received for publication September 20, 1905.

† Presented before the Manila Medical Society April 4, 1905.

‡ In Manila (1903) out of 1,623 rats examined, one-sixteenth of 1 per cent were found infected with plaguelike bacteria.

[1] *Centralbl. f. Bakt.*, 1904, 36, p. 127.

[2] *Zeit. f. Hyg.*, 1903, 45, p. 450.

dogs, jackals, snakes, etc., without the presentation of sufficient evidence in support of the statements.

BACTERIOLOGICAL EXAMINATION.

The rat was an adult male *Mus rattus.** No fleas or lice were found, but it was covered by a large number of red ants; slight edema in subcutaneous tissues of chest; superficial glands not enlarged; peritoneal cavity normal; spleen slightly enlarged; liver congested, and on its surface numerous pin point sized, whitish areas of necrosis; lungs slightly congested; a little fluid in peritoneal and thoracic cavities. Smears from the liver and spleen, stained with Wright's modification of the Romanowski,[1] showed numerous bi-polar staining rods. (See Fig. 1.) All bacteria in preparations from the spleen and liver gave up the stain in Gram's method. Successive stroke cultures were made from the spleen and liver on +1 agar† slants and kept at 36–37°. In 24 hours quite a number of colonies had appeared. These reached a diameter of 2–4 mm. in 48 hours and were not viscous when touched with a platinum needle. As cultures from these produced indol in broth, coagulated milk, and fermented dextrose, levulose, lactose, and saccharose with gas production, they were discarded as agonal or post-mortem invaders of the colon group. Besides these there developed on the slants from the spleen a few colonies of a diplococcus which closely resembled the variety of

*It is generally accepted that the rat is the original host of B. pestis; and however interesting it may be to know the percentage of rats infected during an epidemic in any locality, the importance of knowing what *kind* of rats are chiefly to blame is becoming more apparent every day. Bruce Skinner has presented evidence which seems to point to the complete or partial immunity of the Norway rat (*M. decumanus*) to naturally acquired plague, and has suggested that, through its successful antagonism to the long-tailed rat (*M. rattus*), it has played an important part in preventing the spread of plague in Europe and elsewhere. It would be interesting to know if such is the case in America. According to D. S. Jordan, *M. decumanus* was "introduced into America about 1775, and is now the commonest species, having nearly exterminated the next" (*M. rattus*). Bacteriologic examinations by Liston show that *M. decumanus* also carries plague, but, owing to its domestic habits, *M. rattus* is a greater menace to man. See W. G. LISTON, "Plague Rats and Fleas," *Ind. Med. Gaz.*, 1905, 40, p. 43, and "The Rats of India," *ibid.*, 1905, 40, p. 130.

[1] *Jour. Med. Res.*, 1902, 7, p. 138.

†Wilson (*Jour. Med. Res.*, 1901, 6, p. 53) showed that 1 per cent acid to phenolphthalein is the optimum reaction for B. pestis, the rapidity and amount of growth diminishing progressively with the increase of acid or alkali. My media were prepared according to the recommendation of the American committee, except that the reaction to phenolphthalein was adjusted by the addition of sodium hydroxide alone. (See *Bull. 19*, Bu. of Gov't Labs., Manila, P. I., 1904.)

Micrococcus catarrhalis, described by Lyon and myself from a case of chronic dermatitis.[1]

In 48 hours, however, numerous discrete, pin-point sized, transparent colonies could be seen between the larger ones. Under the hand lens these appeared round, raised, and perfectly transparent, exactly like a non-saprophytic growth of B. bovisepticus. Transplants on +1 agar slants were made from these minute colonies and marked Transplant (1) Spleen (d) and Transplant (1) Liver (d), respectively.

Wright's modification of the Romanowski had on previous occasions yielded such beautiful results in demonstrating the bi-polar staining of B. pestis in preparations from animal tissues that I thought it might yield similar results in preparations from cultures. Very thin films (in distilled water) were prepared from the fine colonies described above as Spleen (d) and Liver (d), dried in the air, and stained in the manner usually employed in staining a film of blood. The result was very surprising; the rods took up the methylene blue uniformly, and many of them were surrounded by a well-defined oval, capsular substance which took the eosin stain. (See Fig. 2.)

The demonstration of what appeared to be a capsular substance, taken along with the delay in the appearance of the primary growth from the tissues, brought to my mind the theory concerning the production of defensive capsules by bacteria presented by Dr. Theobald Smith at the St. Louis Congress of Arts and Sciences.[2] So an effort was made to keep a strict account of each transplant of cultures Liver (d) and Spleen (d), with the idea of noting whether the subcultures changed in any particular manner during cultivation, or if the capsular substance increased or decreased in amount, or changed in any way in its microchemical reactions.

The subcultures were made on freshly prepared +1 agar slants (made up in several different lots and varying slightly in reaction and color and perhaps in composition) and kept at 36–37° until growth had become quite evident to the naked eye (24 to 48 hours), when stained preparations were made. The

[1] *American Medicine*, 1903, 6, p. 401. [2] *American Medicine*, 1904, 8, p. 711.

original subculture was then in each instance kept on a shelf at 18–28° and used for inoculating another subculture or an animal or discarded, as the case might be.

Briefly, the history of Liver (d) may be given as follows:

								Liver (d), inoculated from tissue on March 15, capsules with Wright's Romanowski.					
Transplant	(1)	"	"	"	"	"	"	"	17,	"	"	"	"
"	(2)	"	"	"	"	"	"	"	19,	"	"	"	"
"	(3)	"	"	"	"	"	"	"	22,	"	"	"	"
"	(4)	"	"	"	"	"	"	"	26,	"	"	"	"
"	(5)	"	"	"	"	"	"	"	28,	"	"	"	"

As already stated, the tubes inoculated from the rat's liver showed none of the minute transparent colonies after 24 hours' incubation at 36–37°, but a plainly visible growth was present in 48 hours. In the case of Transplant (1), no visible growth was present after 24 hours at 36–37°, with the possible exception of two or three scattered colonies. The water of condensation was allowed to flow over the surface of the agar slant, and in 48 hours numerous transparent, pin-point sized colonies were present. In the case of Transplants (2), (3), (4), and (5), a very delicate confluent growth appeared after 24 hours' incubation at 36–37°; but it must be noted that the successive subcultures were not made at exactly equal intervals of time.

No peculiar changes in the capsular substance were noted. Owing to my departure I was unable to follow this system through any extended period of time. However, a staining from a culture of B. pestis over two years old (described below as No. 15) showed that the capsular substance was not lost with an increase in the saprophytic powers of the culture. (See Fig. 3.)

The number of bacteria showing this capsular substance seemed to vary somewhat in different preparations. As a rule the organisms lying in the better-spread portions of a preparation showed it most plainly, and though sometimes the capsular substance stained evenly with eosin, at other times it would be merely outlined by a reddish-purple halo. Enough work was not done to prove that this was not due to slight variations in technique.

Culture Spleen (d) in the course of a few transplants grew more luxuriantly than is usual with recently isolated cultures of B. pestis, and losing its

viscosity it took on a granular consistency. A very small amount of the first transplant killed guinea-pig 1132 (numerous bi-polar staining rods). As much as 2 mg. of Transplant (7) failed to kill guinea-pigs. As the culture produced indol and fermented sugars it was discarded as overgrown by B. coli.

BIOCHEMICAL CHARACTER OF CULTURE LIVER (d).

Transplant (4) was inoculated into litmus milk, +1 broth, and Dunham's peptone solution; both of the latter contained a small amount of nitrite. The litmus milk was unchanged, and no indol could be demonstrated after five days at 36–37°.

When grown in +1 broth containing 1 per cent of dextrose, levulose, lactose, saccharose and starch (Kahlbaum's), in the fermentation tube, in each instance, at 36–37°, growth appeared only in the bulb and open neck, i. e., aerobically.

In order to make the specificity of this culture more certain, it was compared in several of its more important biochemical characteristics with the following cultures of B. pestis derived from human sources:

Culture 15.—Isolated by myself from spleen, Autopsy 532 March, 1903. Since then it has been transplanted on +1 agar slants eight or nine times and kept in the ice chest. Grows slowly in the ice chest. Growth, at first delicate, is now quite luxuriant and viscous. Pathogenic for guinea-pigs by scarification at time of isolation.

The marked polymorphism and variation in size shown by the same or different cultures of B. pestis is well illustrated on comparing Fig. 2 with Figs. 1 and 3.

Culture 26.—Is identical with Culture 15, excepting that the virulence of this particular strain was kept up by passage through guinea-pigs until about three months ago. Since then it has been kept in the ice chest. Killed guinea-pigs regularly, by scarification, in four or five days. It has been used by Dr. E. H. Ruediger in immunizing Horse 12 (*vide infra*). Growth is quite luxuriant. Preparations from +1 agar slants show capsules with Wright's Romanowski.

Culture H.—Isolated from a human plague case about February 1905 by Dr. Herzog. Its growth is only moderately

luxuriant. Preparations from +1 agar slants show capsules with Wright's Romanowski.

1. *With regard to the production of involution forms on fresh +1 agar slants containing 2 or 3 per cent of Kahlbaum's chemically pure sodium chloride (proved free from nitrates or nitrites).*—All of these cultures produced an extremely delicate growth after 24 hours at 36–37°. Stained preparations showed large sinuous and clubbed forms which were very much alike throughout the series of cultures. (See Fig. 4.) The use of 2 or 3 per cent of the sodium chloride made no appreciable difference in the character of the growths or polymorphism shown by any particular culture.

2. *With regard to the formation of stalactites in oiled broth.*—A thin layer of cocoanut oil was placed in +1 broth, the tubes sterilized at 120°, inoculated, and kept in a perfectly quiet place at 18–28°. Culture 15 produced stalactites in three days; 26 in four days; and H in about a week. Liver (d) Transplant (2) scarcely grew at all in the broth and produced no stalactites during 12 days' observation.

A number of workers have recorded an inconsistency in the production of stalactites shown by cultures of B. pestis. Theoretically one should be able, by artificial selection, to train such a culture of B. pestis to produce stalactites, just as it is possible to train cultures of cholera, diphtheria, etc., to produce pellicles in broth cultures.[1] According to Lieut.-Col. Bannerman, Director of the Plague Research Laboratories, Bombay, the stalactite growth never fails to appear when flasks of broth containing a drop or two of "ghee" (clarified butter) or cocoanut oil are "seeded" with material taken directly from fresh plague buboes.

3. *With regard to their agglutination with antiplague serum.*—The serum of Horse 12, immunized against culture 26, was kindly furnished by Dr. E. H. Ruediger of the Serum Institute. The cultures, grown for three days on +1 agar slants at 36–37°, were suspended in 0.85 per cent sodium chloride solution, and the suspensions then brought to approximately the same density by further dilution with the salt solution. The serum was diluted

[1] See *Bull. 19*, Bu. Govt. Labs., Manila, P. I., 1904.

with the same salt solution, and one c.c. of each dilution of the serum added to one c.c. of the culture suspensions in small test tubes and kept at 36–37°. The controls made up with salt solution alone were kept under the same conditions. The results are shown in the following table, where + indicates complete precipitation, ± precipitation with a clouded fluid above, and — no precipitation.

Dilution of Serum.....	1/10		1/100		1/200		1/400		1/1000	
Hours at 36–37°.....	16	24	16	24	16	24	16	24	16	24
Culture 26	±	+	−	±	−	−	−	−	−	−
Culture 15	±	+	−	±	−	−	−	−	−	−
Culture H	±	+	−	±	−	−	−	−	−	−
Culture Liver (d).....	−	±	−	−	−	−	−	−	−	−
Culture Spleen (d)*....	+	+	±	+	±	±	±	±	−	−

Controls all negative throughout.

In another test with the same series of cultures, Culture Liver (d) was completely precipitated in 24 hours at 1:10 and 1:20 dilutions.

INOCULATION EXPERIMENTS.

The pathogenicity of the cultures isolated was tested upon a number of guinea-pigs and wild rats, but I will insert only a few protocols to demonstrate the pathogenicity of Culture Liver (d).

1. *Guinea-pig 1129.*—Abdomen washed, shaved, and scarified with a sterile needle. Rubbed in a small amount of the growth of very minute colonies on the original +1 agar culture from liver of rat. (Colonies 48 hours old.) On fourth day slight inflammatory reaction about site of scarification; no enlargement of inguinal glands. On seventh day local reaction increased, raised, reddened: no palpable enlargement of inguinal glands. On ninth day very sick and listless, eyelids gummed together. Chloroformed.

Post mortem.—Oval thickened area of infiltration which is in the right lower quadrant of the abdomen; right inguinal glands 6–8 mm. in diameter and imbedded in an area of gelatinous edema; on section glands show pink and yellowish caseous contents; spleen five or six times normal size, soft, and contains many yellowish nodules 2–3 mm. in diameter; liver congested and contains many nodules as in spleen; kidneys congested; lungs show irregular areas of consolidation which show a reddish-gray surface when cut; no hemorrhages.

*Since this Culture Spleen (d), as stated above, was overgrown by a member of the colon group, this agglutination with antiplague serum might seem remarkable were it not for what we know concerning the absorption of agglutinins for various strains of B. coli from the intestinal tract during the course of certain fevers, e. g., typhoid, or during active immunization with various cultures (See Park and Collins, *Jour. Med. Res.*, 1904, 12, p. 491).

Smears from the skin wound, inguinal glands and lungs showed numerous polar staining rods (many of which were involution forms). No bacteria were seen in the preparations from the spleen and liver (perhaps because they were shut up in the caseous foci which were not broken up in obtaining material for the smears?).

Cultures on +1 agar slants kept at 36–37°. Those inoculated from the local skin wound and liver showed numerous very minute transparent colonies after 24 hours. A loop of blood from the heart gave no growth. A few colonies appeared in 48 hours on slants inoculated from the spleen. Bacteria from these colonies gave the capsule stain with Wright's Romanowski.

2. *Guinea-pig 1131.*—A very small amount of the same culture used in inoculating Guinea-pig 1129 was injected into the peritoneal cavity of this animal. Chloroformed in a dying condition on the seventh day.

Post mortem.—Where the needle entered in the lower left quadrant of the abdomen is a yellowish, caseous, subcutaneous, and intramuscular nodule about 4 mm. in diameter; inguinal glands on left side enlarged, congested, surrounded by a gelatinous edema; right inguinal glands somewhat enlarged; axillary glands apparently not affected; liver congested and shows a few pinhead sized caseous areas; spleen enlarged to 2½ in. by 1 in. by ¼ in., soft and filled with irregular, yellowish-white, caseous areas; caseous nodules along sternum; lungs congested; few pea sized, caseous nodules in abdominal cavity (necrotic glands?); colon greatly thickened, mucosa covered by bloody mucus and contents mucoid with streaks of blood. No hemorrhages.

Smears.—Numerous polar staining rods found in caseous nodules, liver, and spleen. Fresh preparation from colon shows a large number of amebæ-like bodies, non-motile but containing red blood cells and fragments of red blood corpuscles, a few trichomonas, and an organism which looks like the *Megastoma entericum.*

Cultures.—The organism was recovered on agar slants from the heart's blood, spleen, and liver. Time when growth on agar slants appeared not noticed. (N. B.—Tissues and cultures from the guinea-pig's colon were turned over to Dr. Musgrave and Dr. Woolley, who will report on the case if it should prove to be one of spontaneous amebic dysentery in a guinea-pig.)

3. *Rat. 3.*—Large adult male (*Mus decumanus?*). Received intraperitoneal injection of a small amount of Liver (d) Transplant (2) suspended in 0.8 per cent salt solution. Dead in less than 48 hours.

Post mortem.—No fleas or lice found; congestion and swelling of the spleen; congestion of the liver; double, clear, serous hydrothorax; atelectasis of the lungs; no hemorrhages.

Smears from liver and spleen show large numbers of polar staining rods. Rods comparatively fewer in serous fluid, where they occur singly and in chains of 6–8 individuals.

Cultures.—The minute colonies from spleen show capsulated rods when stained with Wright's Romanowski. Time when growth appeared on agar slants not noticed.

4. *Rat 4.*—Adult female (*Mus decumanus?*). Fed on some bread soaked in well-clouded emulsion from which Rat 3 was injected. It remained well during two weeks' observation.

GENERAL CONSIDERATIONS.

It seems to be entirely too soon to speculate on the significance of the production of this capsular substance on nutrient agar. Several questions present themselves: Is it really an integral part of the bacterial cell? Will all bacteria show it, or is it only produced under certain conditions by particular groups of pathogenic bacteria? Is it produced in fluid, as well as on solid, media? If it cannot be demonstrated in animal tissues by the same technical methods, does this fact partly answer the question: "Do the parasites act differently when posing for us in the culture tube from the way they behave in the animal body?"

I do not believe that this capsular substance could be agar precipitated about the bacterial cell, for with this thought in mind I was very careful to touch only just the surface of the growth with the platinum needle; and then the size and shape of the capsule is too uniform to make one think of artifacts.

Through the kindness of Dr. Paul G. Woolley I was able to test this method on a culture of B. bovisepticus recently (48 hours) isolated from a tumor-like growth in the lung of a Chinese bull. This culture also showed a delay in the appearance of the primary growth on +1 agar. A very clearly defined, capsular substance surrounds the rods, as shown in Fig. 5. It is interesting at least that, after fixation with methyl alcohol, one part of the organism should take the nuclear, and the other the protoplasmic, stain. No attempt was made to stain the capsular substance on bacteria in fluid cultures. Even prolonged immersion failed to stain capsules on B. pestis in smears from inoculated animals, though, as is well known, they often appear surrounded by an unstained halo.*

The particular portion of Dr. Theobald Smith's article which suggested the above work I may quote as follows:

*Capt. S. R. Douglas, R.A.M.C., of St. Mary's Hospital, London, informs me that capsules may be stained with Leishmann's dye, on B. pestis, in smears from the buboes of rats inoculated by the cutaneous method. Here some of the rods appear to be enveloped in a capsule stained pale blue, the bacilli themselves being almost black. "This capsule-like appearance was far more frequently found in wipes made from the buboes of rats than in those made from the spleens, but it was occasionally found in spleen wipes. I was never able to satisfy myself whether this appearance was found more frequently in partially immunized rats or in normal control rats, as the staining of these capsules was uncertain."

I am inclined to believe, however, that among the problems of the future will be the elucidation of still another mechanism for the protection and escape of the microorganism. It is highly probable that in a certain number of species of bacteria after the active vegetative stage a latent stage follows, during which the parasite which has escaped destruction provides itself with some protective envelope which also aids it in its passage to a new host. This envelope, which may be some species of substance not recognizable with the microscope, or which may be represented by the capsules in some groups, may be a defensive body of the parasite stimulated to overproduction by the antibodies of the host. This body also interferes with the metabolism of the microbe, and thus acts in the double capacity of stopping the disease and protecting the microbe at the same time. This hypothesis suggested itself to me while endeavoring to account for the peculiar behavior of tubercle bacilli under cultivation.

It is well known that tubercle bacilli from the diseased tissues of cattle grow very slowly and then only upon certain culture media such as blood-serum. After several years of continuous cultivation they multiply vigorously in glycerin bouillon, and can hardly be distinguished in appearance from those human varieties of the bacillus which grow richly from the first or second transfer. There seemed to be no justification to assume that the bacillus had completely changed its metabolism under artificial cultivation. The more rational hypothesis seemed to be the one which assumed the existence of some protective substance only slightly acted upon by the serum, not at all in glycerin bouillon, and therefore a hindrance to multiplication. After repeated transfers, this protective substance was slowly lost, either through a selection of bacilli, or absence of stimulation on the part of the host, or both causes combined, and the growth became as luxuriant as with the more saprophytic human varieties. It is obvious that each group or species of bacteria would have its own special protective device depending upon original capacities of the species which would be gradually developed in power and efficiency with the perfection of parasitic relations.

The formation of protective or defensive coverings, the strengthening or modification of the cell wall, or the secretion of defensive fluids, would account for certain phenomena, which are familiar to bacteriologists, much better than the current theory which bases parasitism exclusively upon toxin production, active or passive.

In cultures we should expect a loss of power to form protective substances because the antibodies are absent. Hence the universal tendency toward the reduction and final loss of virulence with apparently the metabolic and vegetative activities unchanged, and the frequently observed regaining of virulence by passages through a series of animals.

In my own experience the delay in the appearance of the primary growth of certain cultures (B. mallei, B. pestis, and certain members of the Hemorrhagic Septicemia Group of Hueppe) occurs only when bacteria are transferred either directly, or

through an experimental animal, from a naturally acquired form of disease to such an entirely new physical and chemical environment as is furnished by an agar slant; in which case it may be assumed, according to this theory, that the microorganisms are descended from an infinite number of ancestors especially adapted for growth in animal tissues.

But if such an organism once thoroughly "adapted" for growth on nutrient agar is inoculated into an experimental animal and then recovered from the tissues of this animal, here it will be seen that the time element plays an important part; for if the animal dies before the microorganism loses its newly acquired modification, the culture inoculated upon agar slants usually appears within 24 hours.

It is interesting to note that Culture Liver (d), though but one "generation" removed from the tissues of *Mus rattus*, produced a chronic disease in guinea-pigs, as shown by the marked reaction on the part of the tissues of the inoculated animals, while in the case of Rat 3 an acute general infection resulted. Cultures derived from human sources show some variation in their pathogenicity for guinea-pigs, but as a rule recently isolated cultures of B. pestis, when rubbed into a scratch on the abdomen of guinea-pigs, produce septicemia and death in four or five days with little or no tissue reaction apart from the local reaction, lymphadenitis, and splenic tumor. Unfortunately I had not time to conduct a more comparable series of animal inoculations which might help to elucidate a question which seems to me to be of epidemiological interest, i. e.: Are strains of B. pestis which have been adapted for growth in the tissues of the rat for perhaps an infinite number of "generations" as infective for other animals, e. g., guinea-pigs, monkeys, and man, as strains derived from human sources?

DESCRIPTION OF PHOTOMICROGRAPHS.

(Taken by Chas. Martin, Photographer, Bureau of Government Laboratories.)

All the preparations were stained by Wright's modification of the Romanowski. The magnifications were determined by measuring the projected image of a 1/100 mm. stage micrometer. Figs. 1, 2, 4, and 5 are reduced $\frac{1}{6}$: Fig. 3. $\frac{3}{13}$.

FIG. 1.—Smear from the rat's liver, showing the bi-polar staining of B. pestis, × 720.

FIG. 2.—Liver (d), Transplant (3), 24 hours at 36–37° on +1 agar slant, × 720.

FIG. 3.—Plague 15, 24 hours at 36–37° on +1 agar slant, × 700.

FIG. 4.—Liver (d), Transplant (2), 24 hours at 36–37° on +1 agar slant containing 3 per cent of chemically pure sodium chloride, × 720. By comparison with Figs. 1 and 2 it will be seen what a relatively enormous size these involution forms may attain.

FIG. 5.—From a culture of B. bovisepticus, 48 hours on +1 agar slant at 36–37°, × 720.

PLATE 19.

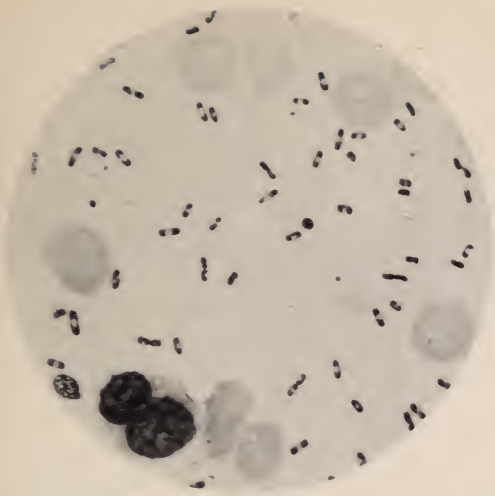

FIG. 1.

FIG. 2.

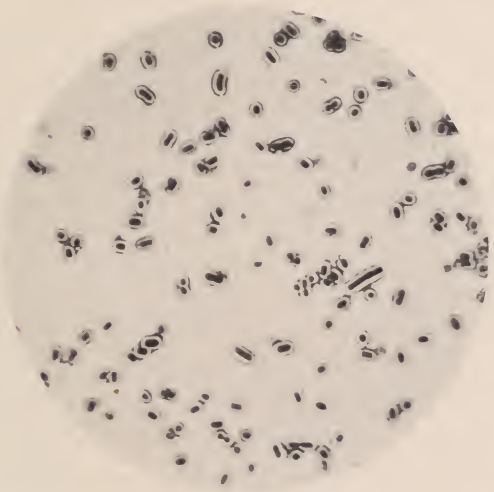

FIG. 3.

FIG. 4.

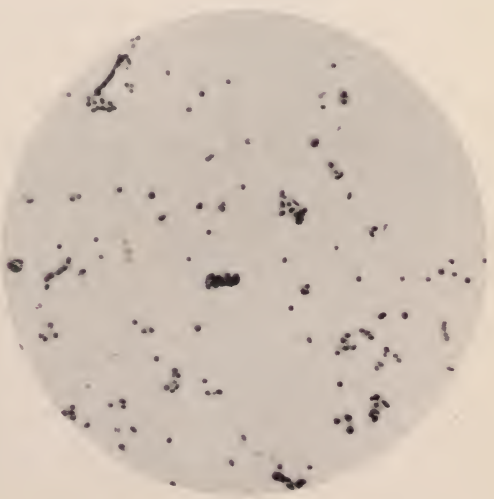

FIG. 5.

SOME OBSERVATIONS ON ACUTE ASCENDING PARALYSIS.*†

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THERE is no need of reviewing the literature of the so-called Landry's paralysis, as that has recently been done by Gordinier[1] and Mettler[2] in this country and Buzzard[3] in England. However, there are some articles too recent to have been reviewed by them. Rolly[4] reports seven cases observed in the medical clinic of Leipzig in the course of the last 15 years and concludes that Landry's paralysis is not a clinical entity but a symptom complex occurring in certain cases of acute polyneuritis, and that cord changes, when they occur, are due to an ascending neuritis reaching the cord. That such a view is untenable has already been pointed out by Mönckeberg[5] who reported the case of a girl of 12 years with typical clinical course and the post-mortem findings of an intense acute poliomyelitis with hemorrhages, while the changes in the peripheral nerves were purely degenerative and readily explained by the destruction of many ventral horn cells. The frequent positive bacteriologic findings, and particularly the finding by Buzzard in such a case, of a diplococcus producing paralysis in rabbits will undoubtedly cause more stress to be laid by future observers on thorough bacteriologic examination and experiments on animals. Babonneix[6] produced an ascending paralysis in dogs by injecting intravenously either the attenuated cultures or toxines of diphtheria and found necrotic foci and diffuse cell changes in the cord and disseminated peripheral neuritis, in one case also fatty degeneration of the muscles. He concludes that the diphtheritic experimental paralysis always presents the same evolution

*Received for publication June 25, 1905.

†Read before Chicago Pathological Society, October 10, 1904.

[1] *Albany Med. Annals*, 1904, 25, p. 56.

[2] *Jour. Am. Med. Assn.*, 1904, 42, p. 1267.

[3] *Brain*, 1903, 26, p. 94.

[4] *Mün. Med. Wchnschr.*, 1903, 50, p. 1283.

[5] *Ibid.*, p. 1958.

[6] *Archives gén. de Méd.*, 2, 1903, p. 3201.

and the clinical picture of acute ascending paralysis as described by Landry, and that it seems to be attributable to central lesions.*

Through the courtesy of Dr. Barber, recently of the Cook County Hospital, and of Dr. Le Count, pathologist to this hospital, I was privileged to attend the autopsy and secure material for histologic examination in the following case.

CLINICAL HISTORY.

The patient, an American roofer, aged 24 years, was admitted to the Cook County Hospital, September 8, 1903. He was sent in by Dr. D. F. Grasse, who kindly gave the facts of the previous history of the patient and also aided in securing the autopsy.

The patient had the usual diseases of childhood. Gonorrhea at 17.

Present illness.—The onset was gradual. September 2, he complained of headache and some backache, was better the following morning, but worse in the afternoon. He continued to work until the afternoon of September 5, when he became dizzy and was afraid he would fall off the roof. There was severe pain, mainly in the abdomen. He took hot whiskey before going to bed, and perspired profusely, but could not sleep on account of pain, and walked the floor all night. September 6, he first bought a porous plaster, then went out to see a physician. He slept two hours during the night. There had been severe pains in the legs all day. The following morning, September 7, the pain ceased in the left leg, which became totally paralyzed. The pain diminished in the right leg, which became paralyzed early in the morning of September 8. On this day he was admitted to the hospital. The arms began to become stiff and weaker, the right more so than the left. The muscles of the neck also became weaker. Urinary retention commenced September 7, necessitating the use of a catheter. The bowels had been regular. The appetite was lost.

Physical examination on admission September 8.—Young man, medium stature, fairly well developed. Lies quietly in bed. Cannot move lower extremities at all. Pulse of good volume and regular.

Sclerotics slightly congested. Pupils small, but equal, and react normally. Ocular movements normal.

Tongue thickly covered with yellowish-white coat. The chest is long and deep; symmetrical; sternum slightly retracted. Pectoral muscles prominent. Breathing almost entirely diaphragmatic.

*Just as this article is going to the press one by SCHMAUS (*Ziegl. Beitr.*, 1905, 37, p. 411) has appeared in which he reports a case with the clinical picture of Landry's paralysis, beginning with disturbance of the muscles of respiration. The autopsy revealed a severe infiltrative and hemorrhagic myelitis with extensive destruction of the ganglion cells; the medulla oblongata was also involved. The trunks of the peripheral nerves were normal, so Schmaus excludes the possibility of a primary neuritis. The colon bacillus was obtained from the blood, spinal cord, tonsil, and a cervical lymph node. A few streptococcus colonies were obtained from the tonsil and spinal cord. Schmaus regards the case as one of infectious, or toxic-infectious, myelitis, and bulbar myelitis which followed a comparatively mild angina, and adds that he knows of only one other case of Landry's paralysis following an angina, namely one reported by Hochhaus.

Pulmonary resonance good throughout.

On taking a deep breath, the abdomen becomes markedly prominent, with but slight motion of the chest walls, and on expiration there seems to be a sudden jerk in the movement of the diaphragm.

Heart dulness, vertically, from third to sixth rib. Laterally, mid-sternal line to just within nipple line. Apex beat in normal position. Sounds clear.

Splenic dulness extends to ninth rib in posterior axillary line. Spleen is not palpable.

Upper border of liver is at fifth interspace in nipple line. Lower border of dulness 3 cm. above costal arch. Not palpable.

No distention of abdomen. Breathing almost entirely abdominal.

Phimosis present. Discharge of thick, yellow, purulent material from urethra.

Sensation is entirely normal. Patient can tell what portion of the body is touched, and whether it is a prick or a touch. Heat and cold are distinguished without hesitation at any point of the lower extremities. In the upper extremities sensation is unimpaired. Movements are possible but quite feeble, more marked in left arm. The extensors are more involved than the flexors. Pronation and supination are of about equal strength. The hand grasp is very weak, slightly more marked on the right side.

Muscles of neck very weak. He cannot lift his head from the pillow.

The lower extremities lie entirely helpless. Motion entirely lost, but on close inspection a slight twitching of some of the muscles can be seen, most prominent in the left gastrocnemius.

Reflexes.—On stroking the bottom of the left foot a slight response is elicited, the great toe being slightly extended. All other reflexes in the lower extremities are lost. The scrotal and abdominal reflexes are also lost. The left pectoral muscles and the muscles of the neck, especially the trapezius, still respond slightly. Of the scapular muscles the supraspinatus has the most marked reaction. The corneal pharyngeal reflexes are intact. The patient can no longer control the sphincter of the rectum and is unable to bear down.

On admission the temperature was 101°, pulse 112, respiration 28.

At 5 P. M., on the same day the following note was made: The patient cannot use the right deltoid, and cannot rotate the right arm. Rotation of right forearm is almost entirely absent. Flexion of right hand good, extension imperfect, especially of little finger. Flexion and extension of forearm weak and incomplete, and as there is no rotation of the upper arm, the forearm drags over the chest. The arm cannot be abducted and adduction is extremely imperfect. The left arm can be partially abducted, and completely adducted and rotated. Flexion and extension of hand and fingers is good.

The posterior cervical muscles are somewhat hypertonic. The trapezius works normally.

In the evening a permanent catheter was inserted, and at 10 P. M. the following record was made:

The right arm cannot be raised. The fingers can be moved. The grip is very slight. The left arm can be raised. The grip is getting much weaker. On deep inspiration the diaphragm is quite jerky. The abdomen is quite distended. He complains of nasal obstruction.

During the night there is a record of profuse perspiration, and of difficult breathing due to nasal obstruction. The urine collected to the amount of 600 c.c. There was no bowel movement.

During the next day, September 9, the arms are recorded as growing weaker, and the fingers of the right hand as being numb. The urinalysis was as follows: yellow; acid; sp. gr. 1022; no albumin, sugar, or casts; pus present.

Blood examination: reds 5,660,000, leucocytes 13,000, hemoglobin 95 per cent.

At 5 P. M. the following note was made:

"The left hand, wrist, and fingers can be flexed and extended, except the little finger. The right hand cannot be extended at all, but can be flexed. The arms and shoulders cannot be moved on either side.

There is pain in the chest on taking a deep breath. During the 24 hours from admission, the pulse rate has been between 80 and 100, the temperature between 100° and 101.2°, and the respirations 28 to 42."

On the morning of the 10th he felt better, but breathing seemed more difficult. Six hundred c.c. of urine had collected; no bowel movements. At noon Dr. Barber made the following record: "Less motion in left hand and fingers. On the right side there is no extension, but slight flexion is still possible. Sensation slightly dulled on trunk and lower limbs. Left leg stiff."

At 4 P. M.:

"Motion of hands almost entirely gone. Only a slight flexion of left fingers remains. The eyes are rolled up. The patient is still rational and can talk."

During the second 24 hours, the pulse rate was 80–100, temperature 99.8°–100.4°, respirations 22–36.

At 4 P. M. on the 10th, a lumbar puncture was made and 10 c.c. of slightly turbid fluid removed. Cultures were made in broth, and smears were stained. All were totally negative. Differential blood count of 250 cells was as follows:

Polymorphonuclear	86%
Small Mononuclear	5.6%
Large Mononuclear	4.8%
Transitional	3.6%

A note is made at 6 P. M. of the patient's talking irrationally, the first record of this kind in the history.

On the morning of the 11th the record is: "Patient cannot move any part of the upper extremities. Breathing is somewhat labored."

During the third 24 hours, 950 c.c. of urine were collected. Pulse was 96–120, temperature 99°–100.2°, respiration 22–32. Several records of a delirious condition were made. On the 12th, after four days without a bowel movement, vigorous purgation was instituted, with no effect till the morning of the 13th, when a large bowel-movement was brought away by an enema. During the fourth 24 hours, 800 c.c. of urine were collected. The temperature was 100°–101°, pulse 112–132, respiration 26–28. During the fifth 24 hours, 400 c.c. of urine were collected, pulse 120–136, temperature 100.6°–103.4°, the rise beginning at midnight on the 12th. The mental condition remained about the same.

From this time to the end, the temperature gradually increased, from 103.6°–106.2°–106.8°–108.8°, the latter being taken 20 minutes before death. The pulse increased gradually to 160, and the respirations to 44. Vomiting occurred on the morning of the 14th. During the last 10 or 12 hours, there was a profuse perspiration, and shortly before death a sudaminal eruption appeared. Death occurred on the 14th at 11:50 A. M.

AUTOPSY.

The autopsy was held three hours after death by Dr. Barber.

The body is that of a well-nourished young man 160 cm. in length. Rigor is extreme; lividity marked behind, and slight in front. The body is still slightly warm. There is a small bed-sore over the sacrum, and a beginning bed-sore over the left scapula. There is a needle wound on the right side, opposite the spinous process of the second lumbar vertebrate. There is slight miliary eruption on the back, shoulders, and neck. A catheter found in the urethra is removed with difficulty on account of a thick coating of crystals.

The abdomen is distended. On cutting through the abdominal wall it is noticed that the blood is very black. The omentum is thin and free, coming within three inches of the pubes. The small intestines are greatly distended. The sigmoid contains scybala. The contents of the small intestines are fluid. The cecum is well in the pelvis; the appendix is parallel to the pelvic brim and free. There are no adhesions about the spleen, liver, or gall-bladder, and no fluid in the peritoneal cavity.

The pleural cavities are empty, and there are no adhesions except over the right apex.

The pericardial cavity contains a small amount of clear fluid; there is a white fibrinous patch on the anterior surface of the heart.

The tongue is coated.

The thyroid is normal.

The larynx and trachea are full of frothy mucus.

The esophagus is normal.

The lungs are dark and less crepitant than normal, especially in the lower lobes. Both lungs float. The right lung weighs 350 grams. There are some torn adhesions over the apex and dark patches posteriorly over the upper lobe. There are some fibrous adhesions in the inter-lobar clefts. Otherwise there is nothing abnormal. The cut section presents a normal appearance except for the presence of fluid in the lower lobe. The left lung weighs 330 grams. The appearance is the same as the right, except for a few emphysematous patches anteriorly.

The heart weighs 315 grams. It is firm to the touch and the apex consists entirely of the left ventricle. There is a sheet of white fibrin on the anterior surface easily removed, leaving a smooth surface and ecchymotic spots under the pericardium. The mitral leaflets are slightly sclerotic; all other valves are normal. The myocardium is dark and firm. There is a white clot in the right auricle, and some white clots adhering to the tricuspid leaflets. Otherwise the heart is normal.

The aorta shows a few sclerotic patches.

The spleen is small, weighing 95 grams. The color is dark red, the consistency slightly increased; otherwise there is no change.

The left kidney weighs 165 grams. It is angular in contour; the capsule strips readily, leaving a smooth surface with prominent stellate veins. The cut surface is dark and bloody. The relation of the cortex to the medulla is as 1 to 3. The mucous membrane of the pelvis is smooth, showing slight congestion of the veins.

The right kidney weighs 115 grams. The capsule is adherent to part of the upper pole. Otherwise it resembles the right kidney.

The ureters show no change.

The bladder contains turbid urine and there is marked congestion of the trigone.

The testicles are normal, but somewhat congested.

The liver comes within 1½ cm. of the costal arch at the right 10th rib and weighs 1470 grams. It is dark in color, the left lobe being darker than the right. It cuts with normal resistance. The markings are fairly distinct and much blood exudes from the cut surface.

The pancreas shows no change.

The stomach is distended and somewhat congested; otherwise normal.

The brain shows no external change except hyperemia of the pia. It was placed in 10 per cent formalin.

The spinal cord shows great redness of the gray matter at all levels.

The tissues of the back are edematous.

Anatomic Diagnosis.—Hemorrhagic poliomyelitis. Purulent cystitis. Hyperemia of all viscera. Pulmonary edema and hypostatic congestion. Localized fibrous pleuritis and fibrinous pericarditis. Slight sclerosis of the aorta and the mitral valves. Edema of the back. Bed-sores over the sacrum and left scapula. Catheter in the urethra.

BACTERIOLOGICAL EXAMINATION.

The blood.—Dr. A. M. Stober, resident pathologist of the hospital, examined the heart's blood, which was collected in pipettes, allowed to coagulate, and placed in the incubator. Small beads appeared in the serum after 24 hours. The growth was found to consist of encapsulated diplococci, arranged in chains. The elements were spherical at first, later somewhat lance-shaped. They stain by Gram's method.

Agar slants.—Slight grayish streaks.

Blood agar.—Growth more luxuriant. Kept alive for three months.

Glucose agar.—Gas produced in 24 hours.

Gelatin stab.—Good growth along the entire length. No liquefaction.

Potato.—Grayish-white, elevated growth.

Litmus milk.—Reduced in 24 hours. Coagulated and pink in 72 hours.

Broth.—A white, flocculent precipitate in slightly turbid fluid forms in 24–48 hours.

Seven days after the autopsy the growth of one blood-agar tube suspended in four c.c. of broth was injected subcutaneously into a rabbit. The animal was very ill for three days, but ultimately recovered. The growths on four agar slants were subsequently injected intraperitoneally, but caused

no marked reaction. Ten days later one c.c. of a broth culture was injected subdurally through a trephine opening without reaction, and later two c.c. into the spinal subdural space without reaction.

The rest of the bacteriologic work was done in the laboratory of Rush Medical Laboratory by Dr. J. A. MacGregor and Mr. E. L. Mattox.

Cord and sciatic nerve.—Emulsions were made in salt solution and various media inoculated. Only the colon bacillus was obtained.

Liver.—Two kinds of colonies developed. One was positively identified as the pneumococcus, the other as the Staphylococcus cereus flavus. The pneumococcus was inoculated into a rabbit, which did not die, but the organism was recovered in cultures from its blood and then killed a second rabbit. This organism was thoroughly studied by Dr. Rosenow, who at that time was making his investigations on pneumonia, and he identified it positively as a pneumococcus.

Peritoneal fluid.—The pneumococcus and Bacillus coli communis.

Spleen.—The Bacillus coli communis.

HISTOLOGIC EXAMINATION.

The spinal cord; cervical region.—Hematoxylin and eosin. The meshes of the pia, particularly at the mouth of the ventral fissure. contain small, perivascular collections of small, mononuclear round cells. The collections, however, are too scant to cause any appreciable thickening in the membrane. The chief change in the white matter consists in the presence of small collections of round cells about the larger vessels. These cells have the size, shape, and staining qualities of small lymphocytes. The vessels are densely packed with red blood cells, their walls unchanged. In places, particularly in front of the ventral commissure on both sides and along the ventral and lateral borders of the ventral horns, there seems to be a slight edema, the texture being loose and an almost homogeneous grayish-blue material present. The dorsal columns appear more compact.

The gray matter shows by far the most pronounced changes. It appears dense and is chiefly made up of dilated vessels engorged with blood and surrounded by extravasated blood, and of various forms of inflammatory cells. Some of the smaller vessels contain many polymorphonuclear leucocytes. The few remaining ganglion cells are so inconspicuous in the midst of the inflammatory tissue as to be easily overlooked on a hasty examination. All parts of the ventral horns are about equally affected, while in the dorsal horns the inflammatory changes are limited to the immediate neighborhood of the vessels. According to their morphology the inflammatory cells may be classified as follows: (1) Cells of the lymphocyte type; numerous. (2) Polymorphonuclear leucocytes; few. (3) Cells slightly larger than small lymphocytes and with less chromatin in their nuclei; only a narrow rim of cytoplasm. (4) Cells with nuclei like the preceding class, and large, round, polygonal, pyramidal, or irregular, granular, unstained, and in places vacuolated, cytoplasm. Frequently distinct small nuclei are seen. These cells are very numerous and are plainly of the type known as "fat granule cells," as they are blackened by the osmic acid in Marchi preparations. (5) Typical plasma cells near the vessels. (6) Oblong cells with almost homogeneous, slightly

stained cytoplasm. The nucleus consists of large, dark chromatin granules; no visible nuclear membrane. These cells are seen near the vessels. (7) Cells with nuclei like class 5, but with large, irregular cytoplasm. (8) Cells without distinct cytoplasm. A rim of granules the size of those usually seen in plasma cells occupy the periphery.

The last three classes probably owe their peculiarities to various forms of regressive changes.

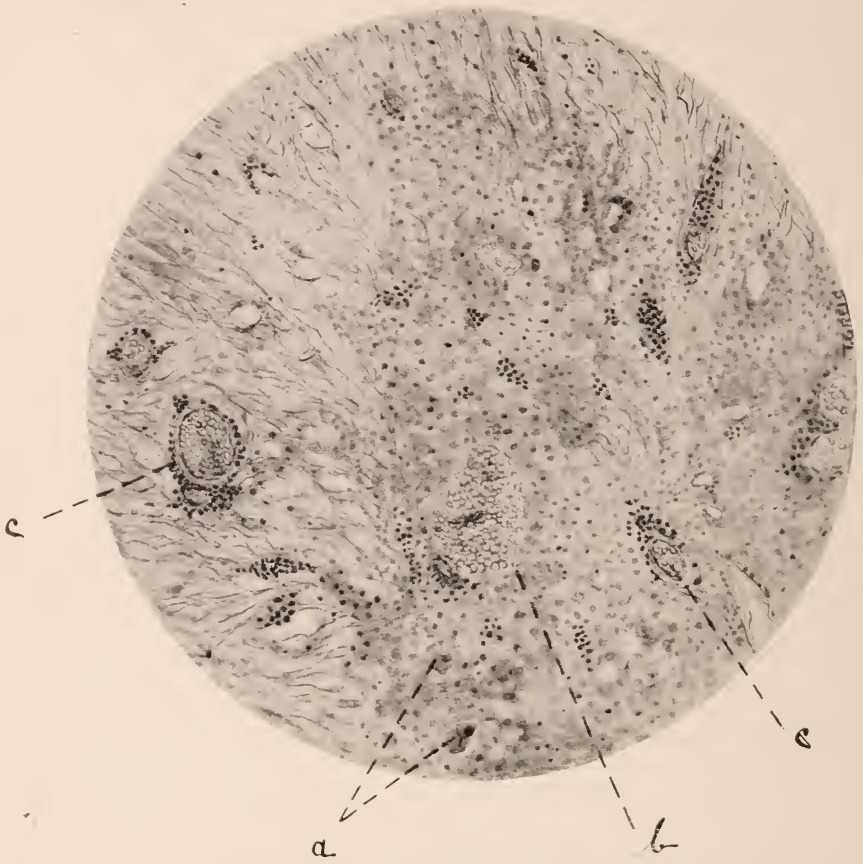

FIG. 1.—Ventral horn from man. Hematoxylin and eosin. Low power.
a. Shadow of ganglion cells.
b. Hemorrhage.
c. Perivascular infiltrations.

Nissl's method (after alcohol hardening).—Only a few ganglion cells are seen in the ventral horns, all showing changes. In the dorsal horns the ganglion cells appear spindle-shaped, with distinct nuclei and nucleoli, and finely granular cytoplasm. On account of the small number of ventral horn cells present and the variety of changes found in them a general description

is impossible. A more trustworthy idea of them may be conveyed by simply describing most of the individual cells encountered in the study of a few Nissl preparations with the oil immersion lens.

Ventral group: (*a*) A cell pyramidal in shape, with a centrally placed nucleus with intact membrane and central nucleolus. In the cytoplasm are large, peripheral heaps of tigroid, no normal Nissl bodies. One process is visible for a long distance. (*b*) Two smaller cells with ragged outlines and devoid of nuclei, with irregular, broken up tigroid masses. (*c*) A rather large, rounded cell, also with ragged outline and encroached on by small, round cells. The cytoplasm contains small, irregular granules. The nucleus is distinct, with a nucleolus, but almost devoid of chromatin. (*d*) A narrow, bulb-shaped cell. Toward the base, the cytoplasm is deep blue, with extremely fine granules. The nuclear membrane is indistinct, the nucleolus distinct. (*e*) An irregularly oblong cell with homogeneous, unstained cytoplasm, and a greatly swollen, finely granular nucleus: no nucleolus.

Ventromesial group: (*a*) A cell resembling *c* in preceding group. (*b*) A large one with deeply stained cytoplasm containing fine and coarse granules, but no typical Nissl bodies. The nucleus is well marked but without distinct membrane. (*c*) A sky-blue homogeneous shadow with a vacuole. (*d*) A greatly shriveled shadow, almost unstained, lying in a wide space. It contains refractile droplets in large numbers. (*e*) Two similar shadows, destruction even more advanced. (*f*) A multipolar cell with distinct outlines. Nucleolus distinct, nucleus not plainly outlined. The cytoplasm presents a fine, spongelike network, with minute circular and oval vacuoles. It is encroached on by round cells. (*g*) An elongated multipolar cell with process visible across two oil immersion fields. No nucleus. A few Nissl bodies are seen in the processes. The cell body possesses irregular tigroid débris only. (*h*) Elongated cell with distinct outlines and distinct nucleus. One process is very long. The cytoplasm is filled with fine blue granules, and from one nuclear pole two rows of larger granules proceed, diverging, to pass one along each side of the cell, then converging toward the beginning of the long process, where they end. (*i*) A long, pyramidal cell with plain nucleus and nucleolus. The cytoplasm is disintegrating and encroached on by round (glia?) cells. A process springing from the apex is visible across an entire oil immersion field. Laterally one comparatively normal multipolar cell is seen, showing some tigrolysis.

A large number of specimens stained with the Gram-Weigert method were carefully examined, but failed to show any organisms.

A few bacilli are seen in Loeffler's methylene blue sections.

No degeneration can be made out in Weigert-Pal sections.

Thoracic region.—The changes are similar to those in the cervical region. The vessels are dilated and engorged with blood. There are large, perivascular collections of round cells in both the gray and white matter. Many vessels, particularly the small ones in the gray matter, contain numerous polymorphonuclear leucocytes. There are no large ganglion cells in the ventral horns, which are occupied mainly by fat granule cells. The latter cells constitute the bulk of the inflammatory cells except close to vessels, where cells of the lymphocyte type predominate. The ganglion cells of

Clarke's columns show no change except a slight tigrolysis. A few thick bacilli are seen in preparations stained by Loeffler's blue. No organisms in Gram-Weigert specimens. There is some diffuse blackening in Marchi preparations. No change in the white tracts can be made out in Weigert-Pal specimens.

Lumbar region.— The picture is similar to that seen higher up. The hemorrhage and round-celled infiltration are most marked in the gray matter, but there are also numerous collections of round cells about the vessels of the white matter, particularly in the lateral columns. The ventral horns are here also mainly made up of inflammatory cells. The few ganglion cells seen are greatly altered, the changes being like those described in the cervical region. Only 17 ganglion cells are seen in one ventral horn in a Nissl preparation 10 microns thick. There is only a diffuse blackening in Marchi's specimens. In Weigert-Pal specimens the lateral columns appear lightened. There are two symmetrically placed lighter areas, both near the periphery, one situated laterally to the most external portion of the ventral horn, the other opposite the dorsal commissure. There also appears to be some loss of fibers in the ventral roots. Here also, Gram-Weigert specimens failed to show organisms.

The *spinal dura* shows no change.

The *spinal ganglia* from the three regions of the cord were fixed in Zenker's fluid and stained by the hematoxylin-eosin, Nissl and Gram-Weigert methods. The ganglion cells only show a moderate tigrolysis involving the whole or central part of the cytoplasm. The nuclei are centrally placed. The usual amount of pigment is present. There are no inflammatory changes in the ganglia themselves, but the adipose tissue outside the thoracic ganglion examined, shows hemorrhage and a large inflammatory focus. The cells are mostly of the small mononuclear type, but there are also many polymorphonuclear cells, some plasma cells, and a few large multinuclear cells (probably endothelial).

The brain.—Pieces from the frontal and motor regions were hardened in alcohol and stained by the hematoxylin-eosin and Nissl methods. Other pieces hardened in formalin were stained with neutral red. There are no definite and constant changes in the ganglion cells. There appears to be some increase in small glia cells. There is no Marchi degeneration in specimens from the left motor cortex, crus cerebri, thalamus, and from the medulla oblongata. Hematoxylin-eosin specimens of the latter show dense collections of small mononuclear round cells about the vessels in every part of the cross sections. There are no inflammatory areas except in the immediate neighborhood of the vessels.

The nerves.— Portions of both vagi, ulnar, anterior crural, and sciatic nerves were treated by the Marchi method. One of the vagi shows no degeneration, the other a moderate degree. The ulnar and anterior crural nerves are distinctly degenerated, the sciatic nerves practically unaffected. The degeneration seems to be the only lesion, as no signs of neuritis can be detected in sections from the anterior crural and sciatic nerves stained by hematoxylin and eosin.

The *thyroid* is hyperemic. The follicles are distended with colloid. Small interstitial hemorrhages are seen.

The *lung* shows marked hyperemia. Many alveoli are filled with blood. There is an increased number of leucocytes in the blood-vessels.

There is a narrow hemorrhagic zone beneath the capsule of the *spleen*. The pulp is hyperemic. Polymorphonuclear cells are numerous both in the Malpighian bodies and pulp. No organisms could be demonstrated in Gram-Weigert preparations.

Small subcapsular hemorrhages are present in the *liver*. Light brown pigment granules are seen in the liver cells of the central parts of the lobules. Gram-Weigert specimens are negative as to microorganisms.

There are slight subcapsular hemorrhages in the *kidneys*. The cells of the convoluted tubules are swollen, their nuclei as a rule intact.

Both the bacteriologic and histologic findings in the preceding case plainly point to a general bacterial invasion with a special localization of the action of the toxic substances in the cord. There can be no doubt as to the presence of pneumococci in the peritoneal fluid and liver. Whether the organism from the blood was another diplococcus or its peculiar behavior on culture media was due to admixture with other organisms must be left undecided. In considering the latter alternative it must be borne in mind that the colon bacillus may assume a diplococcus form (Adami). Assuming the admixture of colon bacilli with pneumococci the appearance of the cultures from the blood may be accounted for. It must also be considered proved that the ascending paralysis in this case was due to primary changes in the gray matter of the cord, and that the degeneration encountered in the motor nerves was a secondary one, as no neuritis could be demonstrated. Only the large nerve trunks were examined. The claim might be made that there had been a primary neuritis of the end ramifications of the nerves, the infective agent then passing rapidly along the nerve trunk to the cord. The elaborate experiments of Homén and Laitinen,[1] who injected streptococci and streptococcic filtrate into peripheral nerves, have demonstrated the rapid migration of such agencies along the lymph channels of the nerve trunks to the cord where severe changes may be set up. However, in their experiments changes were produced in the nerve trunks in the shape of leucocytic emigration, proliferation of the nuclei and of the endoneural cells, etc. In this case the extensive changes in the cord, coupled with the slight simple degeneration of the nerves,

[1] *Ziegl. Beitr.*, 1899, 25, p. 1.

would at once make it appear at least far fetched to consider it one of primary neuritis, and in view of the short duration, the absence of inflammatory changes in the nerve trunks forces the conclusion that the changes in the nervous system first took place in the cord.

In connection with changes in the cord by bacteria and their products, the writer is fortunate enough to be able to relate a rather definite experiment. In a fatal case of carbuncle of the lip a thorough bacteriologic examination by Mr. E. T. Hanley demonstrated the Staphylococcus pyogenes albus as the sole organism present. Cultures were injected subcutaneously into a rabbit. Nodules developed in the vicinity of the point of inoculation but the animal did not become ill until a further inoculation into the blood had been made. About a month after the first inoculation the animal's hind-legs became paralyzed. The following day the forelegs were also paralyzed. It died on the third day. The autopsy revealed a greatly distended urinary bladder. There were no metastatic abscesses.

Pieces of the cord were hardened in alcohol and stained by the Nissl and hematoxylin-eosin methods. No inflammatory changes are present at any level. Nissl specimens from the cervical region show a pronounced tigrolysis in the ventral horn cells. The normal arrangement of the tigroid is nowhere seen. It is broken up into fine particles which again may be clustered together to form larger lumps. In many instances the cell outlines are ragged, the nuclear outlines also destroyed while the nucleoli generally persist. The cells of the dorsal horns are much better preserved, their nuclei being unaffected. Similar cell changes are seen in the thoracic region, while they are less pronounced in the lumbar region. Here there is also marked tigrolysis, but the nuclear and cellular outlines are generally preserved. Marchi specimens from the cervical region show slight blackening on either side of the ventral median fissure and very slight of the lateral columns of both sides. In the lumbar region there is a slight diffuse blackening. Marchi specimens from the right and left sciatic nerves show considerable blackening in both, while no inflammatory processes are seen in hematoxylin-

PLATE 20.

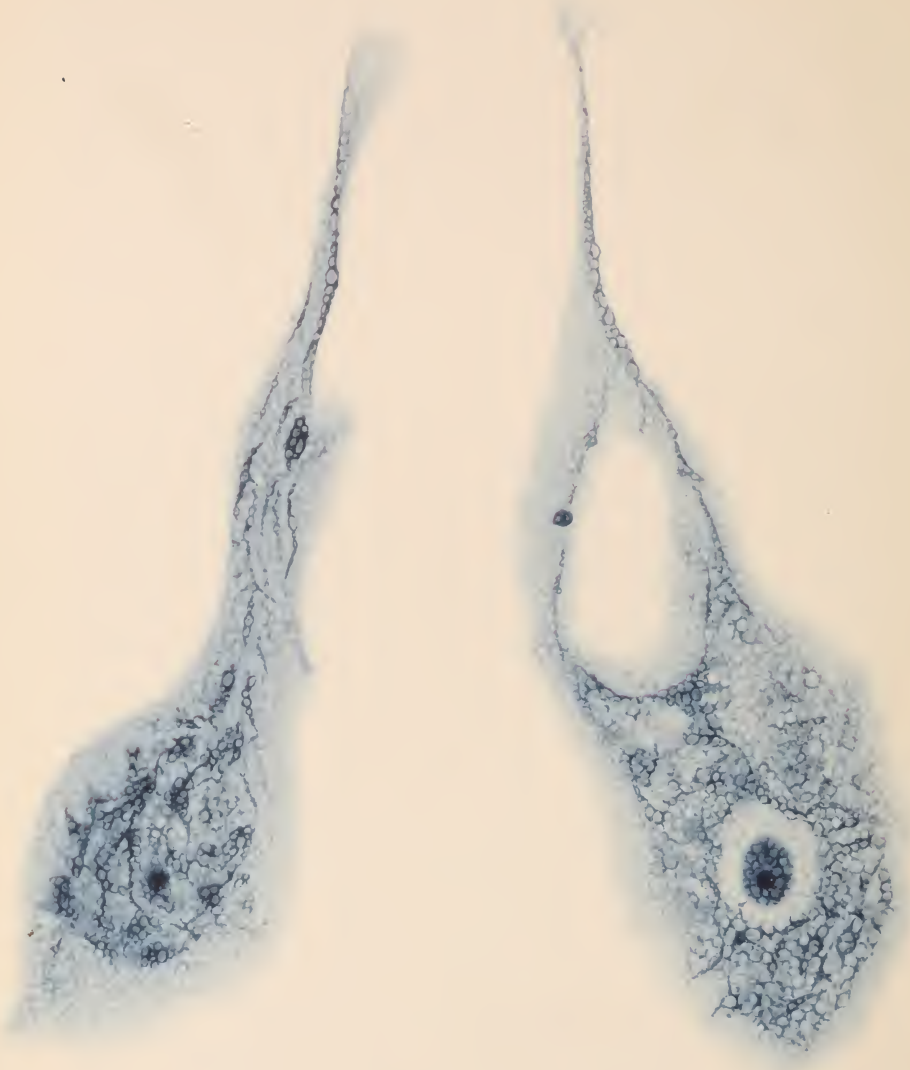

Fig. 1.

Fig. 2.

eosin specimens. Sections from the cortex of the brain fixed in formalin and stained by neutral red show no definite cell changes. There is no blackening in Marchi specimens from the hemispheres.

DESCRIPTION OF PLATE 20.

Figs. 1 and 2.—Ventral horn cells from rabbit. Nissl's stain. Oil immersion 1/12 Zeiss.

STUDIES IN MENINGOCOCCUS INFECTIONS.*

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INTRODUCTION.

A NUMBER of cases of epidemic cerebrospinal meningitis appeared at Cook County Hospital during the spring of 1905. A study of these was begun with particular reference to the bacteriological flora of the nose and throat with a view of determining the frequency of the presence of hemophilic bacteria, these organisms having been studied in several other infections during the winter. To do this complete bacteriological examinations were made of the secretions of the nose and throat upon blood-agar plates and a record made not only of the hemophilic bacteria but also of the various other organisms which appeared on the plates and in smears of the secretions. A bacteriological study was made at the same time of the cerebrospinal fluid and also in all cases but one of the blood. A rather extensive study of the meningococcidal properties of blood and serum of normal persons and of patients with meningitis was also made.

The cases all occurred in Italians recently arrived from Italy. Two of the cases were taken sick on the train coming from New York where they had obtained quarantine papers only a few days previously. This is interesting in view of the fact that an epidemic of this disease was raging in that city at the time. There is no evidence, however, to indicate that these patients were in any way exposed directly to the disease at that place.

A brief report of the individual cases is given below.

CASES.

Case 1.—Italian. Age 19. Taken sick on train from New York. He had a typical attack of meningitis of the fulminant type, dying in less than 24 hours after entering the hospital. The cerebrospinal fluid obtained by spinal puncture was turbid and showed almost exclusively polymorphonuclear leucocytes, many of which contained Gram-negative diplococci. The organisms

*Received for publication September 6, 1905.

were also numerous outside the leucocytes. Cultures showed pure growths of the meningococcus (Diplococcus intracellularis, Weichselbaum). There was considerable expectoration and the sputum was purulent. Smears of the sputum showed many pus cells. The predominating organism was a small Gram-negative bacillus resembling the influenza bacillus. Gram-negative diplococci were numerous. A few Gram-positive diplococci (pneumococci) were present and also some Gram-negative diplobacilli. The pus cells contained some Gram-negative diplococci. Cultures of the sputum showed numerous colonies of hemophilic bacilli, a number of colonies of meningococci, a few colonies of pneumococci, and a few colonies of Gram-positive cocci. Smears of the nasal secretion showed many Gram-positive bacilli resembling pseudo-diphtheria, some Gram-negative biscuit-shaped diplococci, and a considerable number of small Gram-negative bacilli. Cultures gave numerous colonies of hemophilous bacilli, many colonies of meningococci, and some Gram-positive bacilli, probably pseudo-diphtheria. The above material was obtained 12 hours before death. Unfortunately, no blood cultures were obtained in this case.

Case 2.—Italian. Age 19. Taken sick on train from New York. Typical case of acute meningitis, but less severe than Case 1. Cerebrospinal fluid was turbid and contained many polymorphonuclear leucocytes with numerous Gram-negative diplococci within them. The same organisms were also numerous outside of leucocytes. The meningococcus was obtained in pure culture from the fluid. Smears of the sputum showed Gram-positive diplococci, some Gram-positive bacilli resembling diphtheria bacillus and a few large Gram-negative bacilli. Cultures gave numerous colonies of pneumococci, a few of streptococci, none of hemophilic bacilli or meningococci. Smears of the nasal secretion showed a number of Gram-negative organisms suggestive of meningococcus, and a few Gram-positive cocci. A second examination a few days later showed small, Gram-negative bacilli suggestive of influenza bacilli in abundance. Cultures gave, in both examinations, hemophilous bacilli in large numbers, a few pneumococci, and some hemolytic colonies of a Gram-positive coccus (staphylococcus). No meningococcus colonies were obtained on the plates. Blood cultures at three different times remained sterile. This case recovered after several weeks' sickness.

Case 3.—Italian. Adult. Typical case of epidemic meningitis. The meningococcus was obtained in smear and culture from the cerebrospinal fluid. Smears of the nasal secretion gave a few Gram-negative, biscuit-shaped diplococci and numerous chains and pairs of Gram-positive cocci (streptococci). The cultures showed many pneumococcus and streptococcus colonies, and a number of colonies of hemophilous bacilli. No colonies of meningococcus or Micrococcus catarrhalis were obtained in the cultures. The patient died after a few days' illness and a post-mortem was obtained. The meningococcus was isolated from the exudate about the brain and spinal cord. A purulent pericarditis was present, but the exudate showed no organisms in smear or culture. The nasal sinuses contained a large amount of a dirty, greenish mucus which revealed numerous streptococci, and in smear a considerable number of Gram-negative diplococci, but they did not grow on blood-agar plates. The heart's blood was sterile.

Case 4.—Italian. Age 16. Sick four days before entering the hospital. Typical case of meningitis. Recovery after several weeks' sickness. The meningococcus was isolated in pure culture from the cerebrospinal fluid. Smears of the fluid showed the diplococci in abundance. Blood culture gave a negative result. Smears of the nasal secretions (seventh day of disease) showed a large number of pseudo-diphtheria bacilli, many Gram-positive cocci and a few Gram-negative diplococci resembling the meningococcus. Cultures showed a large number of staphylococcus and streptococcus colonies, many pseudo-diphtheria colonies, but no meningococcus colonies or hemophilous bacilli. Three days later a second examination showed nearly pure growth, both in smear and culture, of hemophilous bacilli. No colonies of meningococcus or Micrococcus catarrhalis were obtained. Sputum could not be obtained for examination.

Case 5.—Italian. Age 26. Sick three weeks. Not a severe case. The cerebrospinal fluid had a greenish tinge, was distinctly turbid and contained numerous polymorphonuclear leucocytes, large endothelial cells and a few mononuclears. No organism could be detected in smears of the fluid. In culture the meningococcus was obtained in pure growth. Blood cultures gave a negative result. The patient showed no nasal or throat symptoms. Smears of a nasal swab showed only Gram-positive cocci and cultures showed numerous colonies of Staphylococcus albus. No other organisms were obtained. Examination of throat swabs showed some pneumococci and a few other Gram-positive cocci.

SUMMARY OF BACTERIOLOGICAL FINDINGS.

In the five cases, therefore, the meningococcus was obtained from the spinal fluid in every instance by culture, and observed in smear in all but one. It was isolated from the nasal cavities and the sputum in one case, and in four of the five cases Gram-negative diplococci suggestive of either meningococcus or Micrococcus catarrhalis were seen in smears but not recovered in culture. The blood in the four cases examined gave negative results.

The meningococci from the different cases were identical in every detail. They were Gram-negative, usually in pairs, often single, frequently in tetrads, and never in chains. They grew well in ascites broth and in blood serum and blood agar, but very poorly or not at all in plain broth. No appreciable growth occurred at room temperature. From these characteristics they evidently belong to the Weichselbaum type of meningococcus and not the Jäger-Heubner type. They were all carried through many generations upon various media, three of them being transferred almost daily on blood serum for several months, without

noting any change in their properties, except perhaps a slightly more vigorous growth.

The close resemblance of the meningococcus to the Micrococcus catarrhalis and the gonococcus has been indicated by a number of observers, particularly by Ghon and Pfeiffer,[1] and Libman and Celler.[2]

The Micrococcus catarrhalis is a common organism of the respiratory passages undoubtedly existing frequently as a saprophyte, though often associated with various kinds of inflammatory conditions of this region, particularly in children. It must always be kept in mind in examining nasal and pharyngeal excretions for meningococcus because of the danger of confusing the two organisms. It might, therefore, not be out of place here to indicate briefly some of their distinguishing characteristics. In smears it is practically impossible to distinguish between them. The Micrococcus catarrhalis in the nasal passages and throat is frequently found inside the leucocytes; so far it has never been found in the cerebrospinal fluid. On the whole the Micrococcus catarrhalis is slightly larger and does not vary in size and form to the extent that the meningococcus does; but these are only uncertain criteria for distinguishing them. Culturally certain differences appear quite distinctly. The meningococcus colonies are slightly yellow, have a clear, homogeneous outer zone with a non-granular or very finely granular central portion. The margin is even. The Micrococcus catarrhalis colonies are much more coarsely granular, more opaque and yellowish-brown, while the margin is gnawed. It grows much more abundantly at room temperature than does the meningococcus, at least the Weichselbaum type, and also much better in broth. The two organisms may vary considerably in their pathogenicity for animals, as will be shown later, and this may be of some aid in distinguishing them, but the most positive method of differentiation is the agglutination test with immune serum. The possibility of intermediate forms should be kept in mind, as the biological characteristics of various diplococci frequently found closely resembling these organisms have not yet been given careful study.

[1] *Ztschr. f. klin. Med.*, 1902, 44, p. 274. [2] *Mt. Sinai Hospital Reports*, 1903, 3, p. 542.

The gonococcus, while practically identical with these organisms morphologically, is easily distinguished culturally because of the difficulty of growing it on ordinary media. The gonococcus has not been found in the nasal passages or in the throat, but in bacteriologic examinations of the eye, blood, and joints and even cerebrospinal fluids for meningococci, this organism should be kept in mind, for it may be found in these places.*

The meningococcus obtained from the sputum and nasal secretion in Case 1 was subjected to the most careful tests to distinguish it from the Micrococcus catarrhalis. Culturally it conformed in every detail to the organism isolated from the cerebrospinal fluid of the same case and gave an agglutination reaction with the serum of Case 5 at a dilution of 1:50. A typical Micrococcus catarrhalis obtained from the throat of a case of measles was used as a control. It was not agglutinated by the serum of Case 5.

While it is a very prevalent idea that the meningococcus occurs commonly in the nasal cavities of meningitis patients and even of those not suffering from the disease, examination of the data upon which this notion is based reveals a surprisingly small amount of reliable evidence in its favor. This is due to the imperfect examinations made of the secretions; for the finding of Gram-negative diplococci in smears and on superficial cultural examination has been frequently considered as sufficient to report the presence of meningococci. The reports of many of the cases in the literature are of this kind and hence of little or no value. Councilman[1] recently quoted Lord as stating that there are only four undoubted cases on record where the meningococcus has been isolated from the nasal cavities. This does not mean necessarily that the meningococcus is rarely present in the nasal and pharyngeal secretions, but merely that most of the present data are worthless so far as definitely demonstrating that fact. It is interesting

*It is scarcely necessary to say anything about differentiation of the pneumococcus from the meningococcus, at least the Weichselbaum type, for the two organisms have so little in common, especially culturally, that there can be little difficulty in identifying them. The absence of any zone of hemolysis about meningococcus colonies on human blood-agar plates and the characteristic zone about the pneumococcus colonies serves as a rapid and convenient means of differentiation.

[1] *Jour. Am. Med. Assn.*, 1905, 44, p. 999.

that recent evidence derived from careful cultural and agglutinative tests appears to confirm an older notion founded on absolutely unreliable data. The question as to the frequency of the presence of the meningococcus in the nasal cavities and smears of epidemic meningitis patients and the relation it bears to the frequent complicating rhinitis is therefore, as Weichselbaum says,[1] still an open one.

A number of animal experiments were made with the meningococcus isolated from our cases. On the whole they show rather feeble pathogenicity. When inoculated in sufficient quantity intraperitoneally into guinea-pigs, the animal dies within 24 hours of an acute peritonitis. The exudate contains immense numbers of leucocytes, all of which are packed full of the organisms. Three guinea-pigs of the same size were selected and inoculated intraperitoneally: one with the organism from the cerebrospinal fluid of Case 1, the second with the organism isolated from the nasal secretion of the same case, and the third animal with the same quantity of a culture of Micrococcus catarrhalis isolated from a case of measles. The first two died in 18 hours and the meningococcus was obtained from the pleural fluid and the heart's blood of each. The third animal showed no symptoms whatever. As stated above, this may be used as a means of differentiating the two organisms, but it is known according to Kirchner[2] and to Neisser[3] that occasionally the Micrococcus catarrhalis is feebly pathogenic also, so that such results would not always be reliable.

Attempts were made to inoculate monkeys by swabbing the nasal mucosa and the throat with cultures and also by forcing the material by sprays and swabs high up into the nasal cavities in the region of the cribriform plate. The results were all negative, not the slightest evidence of any symptoms appearing. Two slant cultures from blood serum were also injected directly into the vein of a small monkey without having the least effect.

It is of interest that hemophilous bacilli were obtained in four of the five cases, and that in two of these they were decidedly the

[1] KOLLE AND WASSERMANN, *Handb. der path. Mikroorg.*, 1903, 3, p. 277.

[2] *Ztschr. f. Hyg.*, 1890, 9, p. 528.

[3] KOLLE AND WASSERMANN, *ibid.*, p. 146.

predominating organism. These organisms only grew on hemoglobin media, and morphologically and culturally in every way corresponded to the influenza bacillus. Those from Case 2 and Case 4 had a distinct tendency to form rather long threads. These cases did not show especially marked coryzal symptoms except Case 3, in which there was an abundant secretion of a viscid mucus. There was, however, considerable increase in mucus, which accumulated far back in the nasal cavities and could be obtained on the swab. From what is known of this influenza-like organism it would not be unreasonable to attribute to it such symptoms as the above. A study of nasal secretions of cases of meningitis with especial reference to these organisms should be made, and it may be found that they are concerned in some way with the coryzal symptoms often observed in meningitis.*

Pseudo-diphtheria organisms were obtained in two cases. These are similar to the organisms of this character found frequently on the normal nasal mucosa, and are probably of little significance. The streptococcus was obtained from the nasal cavity in Case 3 in large numbers and in small numbers from the sputum of the same case. These organisms produced a wide, clear zone of hemolysis on the blood-agar plates and occurred in long chains. Gram-positive bacilli resembling staphylococci were commonly found in the nasal secretions, but are probably of little significance, being of the same character as those found so frequently normally.

AGGLUTINATION.

Agglutination tests were made in four cases by the microscopic method. The organism from Case 5 was agglutinated by the patient's serum in dilution of 1:100 in 30 minutes. It was practically complete in one hour. With two normal sera the organism showed no indication of clumping even in pure serum or diluted one-half. The serum from Case 4 agglutinated the organism from Case 2 in a dilution of 1:50 in 30 minutes, and the same organism was agglutinated by its homologous serum in

* It is to be noted that at the time these cases of meningitis appeared there were no cases of influenza in the city to speak of, and hence the presence of these organisms cannot be attributed to a prevalent influenza infection.

the same dilution. Controls with normal serum showed no agglutination whatever.

An opportunity was offered to test the serum of a young man who had had a typical attack of meningitis, from whose cerebrospinal fluid the meningococcus was obtained two years and four months previously. There was distinct agglutination at a dilution of 1:2 and only very slight clumping after one hour at 1:10. Above this no effect was noted.

The data are insufficient to decide as to the time of the appearance of the reaction, but at the ninth day, as shown in Table 1, the reaction is well marked.

TABLE 1.

AGGLUTINATION OF MENINGOCOCCUS.

CASE	TIME AFTER ONSET OF DISEASE	DILUTION				SOURCE OF ORGANISM
		1:10	1:20	1:50	1:100	
2.....	4th week	+	+	+	0	Case 2
4.....	9th day	+	+	+	0	Case 2
5.....	3d week	+	+	+	+	Case 5
6.....	2 yrs. 4 mo.	+	0	0	0	Case 5
Normal serum I..	.....	0	0	0	0	Case 2
Normal serum II.	.....	0	0	0	0	Case 5

The agglutination test may be of great help in the diagnosis of these cases, particularly those in which cultures of spinal fluid give negative results, as not infrequently occurs. In Case 5 in this series the first cultures made from the spinal fluid were sterile, and there was considerable doubt as to the diagnosis in view of the fact that he had been sick over three weeks and was running a chronic, mild course. In a second attempt no organisms could be seen in smears of the fluid, and only by the most careful technique were a few colonies finally obtained in culture. The agglutinative reaction, however, was clearly obtained at a dilution of 1:100 in 30 minutes. In suspicious cases, should no organism be found in the cerebrospinal fluid, it is therefore advisable to try the agglutination test. It is valuable also in distinguishing the epidemic form of meningitis caused either by the Weichselbaum or the Jäger-Heubner type of meningo-

coccus from meningitis caused by other organisms, as the pneumococcus, streptococcus, tubercle bacillus, etc. For Sorgente[1] and others have shown by extensive animal experiments that both types of meningococcus react alike to immune serum, while various other diplococci from different sources and closely resembling the meningococcus are not agglutinated by the serum of animals immunized to the meningococcus.

EFFECT OF DEFIBRINATED BLOOD, BLOOD SERUM, AND CEREBRO-SPINAL FLUID ON THE MENINGOCOCCUS.

Defibrinated blood.—Experiments were made to determine the bactericidal effect of normal blood and blood from patients suffering from meningitis, and also of other fluids upon the meningococcus. The method used consisted in introducing into small

TABLE 2.

EFFECT OF NORMAL BLOOD ON MENINGOCOCCUS.

	At Once	3 Hours	6 Hours	20 Hours	30 Hours
1. Normal Blood I .6 c.c.....	17	71	38,400	∞	....
2. " " " "	112	0	1,952	∞	....
3. " " " "	352	24	192	∞	....
4. " " " "	1,208	3	0	0	....
5. " " " "	2,800	10	150	∞	....
6. " " " "	3,300	6	0	232	....
7. " " II "	960	3	1	10,000	....
8. " " " "	1,500	50	320	∞	....
9. " " " "	1,900	350	3,000	∞	....
10. " " III "	856	5	0	0	....
11. " " IV "	3,120	0	0	0	....
12. " " V "	1,184	0	0	0	....
13. " " " "	20,000	176	55	7,200	∞
14. " " VI "	552	0	0	0	....
15. Dog's Blood "	880	0	0	0	....

NOTE.— ∞ indicates over 15,000.

tubes accurately measured quantities of the material to be tested (blood, serum, spinal fluid, etc.) and this was inoculated with a uniform quantity of a 24 hour culture of meningococcus. Ascites-agar plates (1 part heated ascites fluid to 4 parts plain agar) inoculated with a loop from the tubes were made at once and at varying intervals of time thereafter, usually 3, 6 and 18 or 24 hours. The colonies appearing upon the plates were counted after 48 hours.

The bactericidal property of defibrinated blood from six normal adults was tested on the meningococcus. The results are

[1] *Centralbl. f. Bakt.*, 1905, 39, p. 1.

not uniform, there being considerable individual variation. In blood from two of the six cases the organisms grew abundantly. One of these was tested at six different times and in every instance but one, good growth occurred. In the other four cases no colonies appeared on the plates after three hours. Table 2 shows the number of colonies on the plates at various periods. It is to be noted that in nearly every instance a rapid decrease occurs in the first few hours. This may be due to phagocytosis, which will be discussed in greater detail later on.

The individual variation in the blood from normal persons is interesting in view of the possible relation it may bear to susceptibility to infection.

Blood from three cases of meningitis was tested, and in every instance no growth occurred in the plates after three hours (Table 3). Unfortunately blood was not obtained from any of

TABLE 3.

EFFECT OF MENINGITIC BLOOD ON MENINGOCOCCUS.

		Time After Onset	At Once	3 Hours	6 Hours	20 Hours
1. Men. Blood (Case 2)	.6 c.c.....	5th week	16	0	0	0
2. " " "	.6 c.c.....	6th week	90	0	0	0
3. " " "	.6 c.c.....	6th week	536	7	0	0
4. " " "	.6 c.c.....	7th week	282	1	0	0
5. " " (Case 4)	.6 c.c.....	10th day	40	0	0	0
6. " " "	.6 c.c.....	13th day	1,296	0	0	0
7. " " (Case 5)	.6 c.c.....	3d week	1,400	0	0	0

these cases earlier than the tenth day. It would be very desirable to test the blood at the time of the onset and later in the disease; also to test the blood in fatal cases, for as it happened each of the three cases tested recovered. This also applies to the experiments with meningitic sera given below.

Normal and meningitic sera.—Differing from the streptococcus and pneumococcus, the meningococcus does not grow in normal or meningitic sera, being quickly killed in from one to two hours. Neither will it grow in heated sera (60–65° C. for 30 minutes), though in this the bactericidal power is somewhat diminished. In order to determine this power, varying quantities of the serum were added to ascites broth, in which it grows well, and plates made as usual. The result is shown in Table 4.

Experiments were repeated with a number of normal sera and also with sera from three cases of meningitis with uniform results. The data given in this table are not composite but are from a series made at one time with the same organism, the same normal and immune blood, and the same ascites broth. They represent very typically the results that were obtained with various other sera and other strains of organisms. In this table also is given the effect of heated serum added to washed corpuscles, which it was found furnishes a most excellent medium for this organism.

TABLE 4.

EFFECT OF SERA ON GROWTH OF MENINGOCOCCUS.

		0 Hrs.	3 Hrs.	6 Hrs.	18 Hrs.
1.	Normal Serum .6 c.c.	1,150	0	0	0
2.	" " .4 c.c. + Asc. Broth .2 c.c.	1,280	80	75	0
3.	" " .3 c.c. + " " .3 c.c.	1,800	85	50	560
4.	" " .2 c.c. + " " .4 c.c.	42	336	1,600	∞
5.	" " .1 c.c. + " " .5 c.c.	640	660	10,000	7,000
6.	Normal Serum heated .6 c.c.	1,000	10	0	0
7.	" " " .4 c.c. + Asc. Broth .2 c.c.	1,150	960	820	240
8.	" " " .3 c.c. + " " .3 c.c.	1,200	950	2,200	∞
9.	" " " .2 c.c. + " " .4 c.c.	1,150	1,100	1,800	∞
10.	" " " .1 c.c. + " " .5 c.c.	1,300	1,500	∞	∞
11.	Meningitis Serum (Case 5) .6 c.c.	960	1	0	0
12.	" " " .4 c.c. + Asc. B. .2 c.c.	800	37	12	0
13.	" " " .3 c.c. + " " .3 c.c.	640	90	69	2
14.	" " " .2 c.c. + " " .4 c.c.	672	360	104	27
15.	" " " .1 c.c. + " " .5 c.c.	1,120	960	92	216
16.	Men. Serum heated .6 c.c.	650	0	0	0
17.	" " " .4 c.c. + Asc. Broth .2 c.c.	760	150	2	0
18.	" " " .3 c.c. + " " .3 c.c.	1,100	450	380	6
19.	" " " .2 c.c. + " " .4 c.c.	480	520	460	∞
20.	" " " .1 c.c. + " " .5 c.c.	450	490	1,100	∞
21.	Normal Blood .6 c.c.	1,500	50	320	∞
22.	Normal Washed Corp. + Heated Nor. Ser. = .6 c.c.	2,600	2,800	∞	∞
23.	" " " + Men. Serum = .6 c.c.	900	0	0	84
24.	Men. Blood .6 c.c.	1,440	0	0	0
25.	Men. Washed Corp. + Heated Men. Ser. = .6 c.c.	1,650	1,200	650	10,000
26.	" " " + Nor. Ser = .6 c.c.	1,100	0	0	0
27.	Ascites Broth (1:4) .6 c.c. (Control)	1,500	1,350	15,000	∞

The table shows that the bactericidal effect of normal heated serum is not as great as that of normal unheated serum. This is indicated in two ways. If No. 3 (Table 4) is compared with No. 8, in which equal parts of the heated and unheated sera and ascites broth are used, it is seen that the unheated serum strongly inhibits the development of the organisms while No. 8 is an excellent medium for them. Again by comparing Nos. 21 and 22, the latter, which contains the heated serum, is much more favorable for their growth. This was brought out in a still more striking way with the normal bloods referred to in Table 2, in which the meningo-

coccus did not develop. In every such instance heated normal serum added to the washed corpuscles furnished a most excellent medium for their growth. We may therefore say that the bactericidal power of normal serum is very materially diminished by heating to 60° for 30 minutes.

Comparing normal serum with meningitic serum (three weeks after onset) we see that the bactericidal effect of the latter is greater than the former, and we also note from the table that heating the meningitic serum markedly decreases its inhibitory effect, so that there is little difference between heated normal serum and heated meningitis serum. This is also shown very clearly in Nos. 24 and 25. When the serum of meningitic blood is replaced by the same heated serum the organisms grow very well. Here, however, the question of phagocytosis is also involved, as will be explained farther on. When meningitic serum is added to normal washed corpuscles (No. 23) growth does not occur. This result was obtained with three different meningitic sera. When normal serum is added to meningitic washed corpuscles (No. 26) as a rule no growth occurs, though results of several experiments were not uniform, varying in fact much as the results for normal blood varied.

From the above facts, therefore, we may conclude that the meningococcidal power of meningitic serum is greater than that of normal serum and that this property is diminished by heating to 60° for 30 minutes.

Cerebrospinal fluid.—The bactericidal power of cerebrospinal fluid was also determined in the same manner. Table 5 gives the results obtained and also a control with normal salt solution. The cerebrospinal fluid used was from a case of chronic hydrocephalus; it was perfectly clear and contained no cellular elements to speak of. A second experiment with fluid from a case of uremia gave virtually the same result. No growth occurs in the pure fluid, and only when mixed in about equal proportions with ascites broth does the meningococcus thrive. Heating to 60° for 30 minutes does not change its properties essentially. Compared with normal salt solution it is distinctly more unfavorable for their growth. In fact it acts much like normal heated serum.

TABLE 5.

EFFECT OF CEREBROSPINAL FLUID ON THE MENINGOCOCCUS.

		0 Hrs.	3 Hrs.	6 Hrs.	20 Hrs.
1.	Cerebrospinal Fluid .6 c.c.	1,750	51	2	0
2.	" " .5 c.c. + Asc. Broth .1 c.c.	1,300	1,450	3	0
3.	" " .4 c.c. + " " .2 c.c.	1,456	1,700	640	0
4.	" " .3 c.c. + " " .3 c.c.	1,080	1,300	1,400	4,500
5.	" " .2 c.c. + " " .4 c.c.	1,600	1,600	2,200	8,000
6.	" " .1 c.c. + " " .5 c.c.	1,360	1,500	1,950	∞
7.	Cerebrosp. Fl. heated .6 c.c.	2,200	12	0	0
8.	" " " .5 c.c. + Asc. Broth .1 c.c.	2,200	2,350	1,100	0
9.	" " " .4 c.c. + " " .2 c.c.	2,450	2,500	3,500	0
10.	" " " .3 c.c. + " " .3 c.c.	2,800	3,500	3,400	6,000
11.	" " " .2 c.c. + " " .4 c.c.	2,080	1,950	4,200	∞
12.	" " " .1 c.c. + " " .5 c.c.	1,950	2,100	2,800	∞
13.	Ascites Broth .6 c.c. (Control)	1,500	980	1,600	∞
14.	Normal Salt .6 c.c.	4,400	0	0	0
15.	" " .4 c.c. + Ascites Broth .2 c.c.	1,456	1,072	6,000	∞
16.	" " .3 c.c. + " " .3 c.c.	2,880	3,500	5,000	∞
17.	" " .2 c.c. + " " .4 c.c.	3,040	4,000	3,500	∞
18.	" " .1 c.c. + " " .5 c.c.	3,200	3,500	4,200	∞

PHAGOCYTOSIS.

Beside the bactericidal properties of plasma and serum another factor of importance in explaining the destructive action of blood upon bacteria is phagocytosis. Through the new contributions made to this subject by Wright and Douglas, Hektoen and Ruediger, and others, the details of the mechanisms at work certainly are much clearer and better understood than formerly.

From this point of view a few experiments were made with the meningococcus in order to detect possible differences in the opsonic power of normal blood and meningitic blood. The results are given in Table 6. The method used is that employed by Wright and Douglas, and, briefly, consists in mixing in small tubes definite amounts of the fluids used with bacterial suspension in NaCl solution, incubating at 37° C. for about 10 minutes. Then smears are made and stained with Leishmann's stain and the number of organisms taken up by the leucocytes counted. From 20 to 40 leucocytes were studied and the average number of organisms then computed.

In serum, normal or otherwise, the meningococcus rapidly undergoes a degenerative change. Even in hanging drop this is shown by the granular appearance, as if disintegration were taking place. In stained preparations it is more evident, many of the cocci failing almost entirely to take the stain, and appearing

more like granules than bacteria. This is particularly true of the meningococci which have been clumped in immune serum. The same phenomenon occurs within the phagocytes, as we should expect, under the influence of intracellular digestion and the strong reducing properties of the protoplasm. Leucocytes containing the organisms, especially after about 20 to 30 minutes, appear filled with granules rather than organisms, and this makes counting frequently very difficult and often impossible. It is therefore advisable to make smears in the phagocytosis experiments after about 10 minutes, so as to avoid as far as possible this rapid disintegration.

TABLE 6.

PHAGOCYTOSIS OF MENINGOCOCCUS.

1.	Normal Human Blood I	13.8
2.	" " " II	11.0
3.	" " " III	18.1
4.	Washed Human Corpuscles	0.1
5.	" " " + heated Serum (60° for 30')	0
6.	Meningitic Blood I (13th day of disease)	14.8
7.	" " II (7th week of disease)	11.9
8.	" " III (3d week of disease)	12.2
9.	Meningitic washed Corpuscles	0
10.	Normal washed Corpuscles + Normal Serum	13.0
11.	" " " + Meningitic Serum	11.1
12.	Meningitic washed Corpuscles + Normal Serum	9.9
13.	" " " + Meningitic Serum	11.6
14.	Washed Normal Corp. + Men. sensitized with Normal Serum	5.1
15.	" " " + " " " Meningitic Serum	3.9
16.	" " " + " " " Normal Serum	4.6
17.	" " " + " " " Meningitic Serum	5.0
18.	Washed Normal Corpuscles + Cerebrospinal Fluid*	0.1

As the table shows, the meningococci are readily taken up by the leucocytes of normal blood, while washed leucocytes alone or to which heated serum is added are inactive. No essential difference was observed between normal leucocytes and meningitic leucocytes in their power to ingest the organisms. The individual observations vary considerably, but the differences are such as to be within the limits of experimental error. This holds true not only for the defibrinated blood, but in those experiments

*From case of chronic hydrocephalus.

where meningitic serum was added to normal corpuscles, and *vice versa*, and also in the experiments in which meningococci sensitized with normal and meningitic sera were used.

It is quite evident from the above facts that at least two factors are operative in the destructive action of blood upon meningococci, namely, the bactericidal action of serum, and phagocytosis. For an explanation of the former we may resort to Ehrlich's hypothesis and assume the presence of an amboceptor-complement complex present in normal serum, as evidenced by the decreased bactericidal action of heated normal serum, which we may interpret as due to the destruction of the thermolabile complement. In meningitic serum the amboceptor-complement complex is more active through an increase of the amboceptor element, for heating this serum likewise reduces its antibacterial properties by destroying the complement, and hence the two heated sera act alike.

The question naturally arises, why does the meningococcus not grow in heated serum? Compared with normal salt solution, heated serum is distinctly more bactericidal, and this points to the presence of an antibacterial property independent of the amboceptor-complement complex. This is also indicated from the behavior of cerebrospinal fluid, where little or no change is produced by heating; yet it, also, is distinctly more bactericidal than normal salt solution, and, as stated above, acts much like heated serum. The explanation is unknown. It is certainly interesting that a substance like cerebrospinal fluid, which contains practically no cellular elements and extremely small quantities of albuminous substances, should act so differently from salt solution. It may be a question of reaction or the presence of certain salts. This should be investigated further.

The experiments do not indicate any essential change in the opsonin content of the blood during the course of the disease. However, slight differences would not be easy to detect, for the reason that the organisms are ingested with such avidity by the leucocytes in both normal and meningitic blood, and also because the cocci lose their staining property so rapidly inside the cells that the counts are not absolutely trustworthy.

Reference has already been made to Nos. 24 and 25, Table 4, which show that heated serum added to washed corpuscles affords an excellent medium for the meningococcus. Here we have beside the destruction of the complement the destruction of the opsonin of the serum, and consequently phagocytosis is prevented. This experiment, therefore, shows the combined effect of destroying the two chief bactericidal mechanisms of the blood, and results, as we should expect, in the formation of a favorable culture medium.

It is an interesting point that normal cerebrospinal fluid contains no opsonin, as is shown by the fact that no bacteria are taken up when the fluid is added to washed leucocytes. The opsonin which is present in the fluid in the inflammatory state, as evidenced by the phagocytosis in meningeal exudate, arises therefore as a result of the inflammatory process, probably by the exudation of the plasma through the vascular walls. It appears that, in this disease at least, we have merely a change in the distribution of the opsonin to guard against inimical factors rather than any essential change in the total quantity of opsonin in the blood. This may be capable of a more general application.

From the above facts it follows, theoretically at least, that an abundance of opsonin in the meningeal fluid of the spinal canal would favor phagocytic destruction of the infecting meningococci. In the treatment, therefore, of epidemic meningitis it would not be irrational and might be effective to inject into the spinal canal fresh* normal human serum because of its rich opsonin content and also because such serum in addition has a distinct meningococcidal action. Such a fluid theoretically would be more efficacious than the various antiseptics used, which may not only destroy phagocytosis, one of the most important natural protecting mechanisms at work in the disease, but also the meningococcidal complement in the exudate as well. No attempt has been made so far to treat cases in this manner. The practical difficulty of having the serum reach all parts of the meninges involved appears a serious one to overcome, but not more so than in the case of antiseptics injected into the canal.

*It would be necessary to use fresh serum because the opsonins disappear in the course of several days from the serum. In the ice-box it remains active for a much longer time. (See HORTON, *Trans. Chi. Path. Soc.*, 1905, 6, p. 297.)

Recently Wolff,[1] reasoning from certain observations which he made in growing the meningococcus with the B. diphtheriae and in antitoxic serum, suggested the use of diphtheria antitoxin in the treatment of epidemic meningitis. This treatment has been given trial, particularly in New York, by a number of physicians. Their reports are conflicting, some reporting favorably and others not observing any effect on the course of the disease by this treatment.

Wolff states that meningococci sown into diphtheria antitoxin (I assume untreated antitoxic serum, though he does not so state) show a clear serum after 48 hours and also after nine days, devoid or nearly so of organisms—also that three c.c. of antitoxin added to a 24 hour broth culture cause a precipitate in 24 hours and are devoid of organisms after 48 hours. He does not mention control experiments. I have tested the meningococcidal property of normal horse serum and also of untreated antitoxic serum of 600 units strength, kindly furnished me by Dr. A. P. Ohlmacher of Stearns and Company, Detroit, by the same method used in testing the bactericidal property of human sera, and there does not appear to be any essential difference between them in this respect. Horse serum is less favorable for the growth of the meningococcus than human serum. The experiments mentioned by Wolff are absolutely inconclusive so far as demonstrating any specific action of diphtheria antitoxin in meningitis; and he does not show that antitoxic serum acts in any respect different from any other serum.

RÉSUMÉ AND CONCLUSIONS.

Necessarily conclusions deduced from such a limited amount of material as was available for this study must be considered more or less provisional. The following are thus offered:

1. In five cases of epidemic cerebrospinal meningitis the meningococcus (Weichselbaum type) was obtained in every case from the cerebrospinal fluid and in one case from the nose and sputum by culture. In the other four cases Gram-negative diplococci suggestive of either meningococcus or Micrococcus catarrhalis were seen in smears but not recovered in culture.

[1] *American Medicine*, 1905, 9, p. 775.

2. Hemophilous bacilli were found in four of the five cases, being very abundant in two.

3. Agglutination of meningococci by serum of patients with meningitis occurs in dilution of 1:50 or higher.

4. The meningococcus grows in some defibrinated normal bloods but not in others, there being thus an interesting individual variation. In the blood of three meningitic cases it did not grow.

5. Normal human serum is distinctly bactericidal toward meningococci. This property is increased in the sera of meningitic cases and is diminished but not entirely destroyed by heating to 60° for 30 minutes.

6. Cerebrospinal fluid acts in much the same way as heated serum.

7. In the presence of human serum the meningococci are taken up by human leucocytes.

8. The opsonin content of the blood does not appear to be altered during the course of epidemic meningitis.

9. Normal cerebrospinal fluid does not contain opsonin for meningococci.

I wish here to acknowledge my indebtedness to Dr. Hektoen for suggestions and to Drs. Baum and Weaver, Attending Physicians at Cook County Hospital, for the privilege of utilizing the material upon which these studies were made. I also wish to thank the internes at the Hospital for their many courtesies.

A STUDY OF THE DEJECTA OF NORMAL CHILDREN AND OF THOSE SUFFERING FROM ACUTE AND SUBACUTE DIARRHEA WITH REFERENCE TO B. DYSENTERIAE.*

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UP to the present time, except in a limited number of cases, B. dysenteriae, though widely searched for, has not been found in the dejecta of normal individuals or those suffering from diseases other than dysentery.

Shiga,[1] Flexner,[2] Drigalski,[3] and others, after the examination of a large number of cases, chiefly adults, failed to demonstrate the presence of this organism in normal stools.

Duval[4] states that he found in two instances B. paradysenteriae of the Flexner-Harris type in the normal stools of milk fed infants.

Wollstein[5] in a series of 32 non-dysenteric infants, did not find B. dysenteriae in any instance. In 24 other cases examined at autopsy, B. paradysenteriae was found in the scrapings from the mucous membrane of the intestines three times. Two of these cases gave a history of previous attacks of diarrhea, while the third case had developed a terminal dysentery five days before death.

The term diarrhea is used wherever the exact nature of the attack was not ascertainable; while the term dysentery is used only in those cases where the organism was isolated. The term paradysentery is applied to the types which ferment mannite and differ in their agglutination reaction, but in all other respects correspond to the organism isolated by Shiga as the cause of dysentery.

* Received for publication August 23, 1905.

1 *Centralbl. f. Bakt.*, 1898, 23, p. 599. *Ibid.*, 1898, 24, pp. 817, 870, 913. *Deutsche med. Woch.*, 1901, 27, pp. 741, 765, 783.

2 *Studies from The Rockefeller Inst. of Med. Res.*, 1904, 2, p. 121.

3 *Veröff. a. j. mil. Sanitätswesen*, 1902, 20, p. 86.

4 *Studies from The Rockefeller Inst. of Med. Res.*, 1904, 2, p. 42.

5 *Ibid.*, p. 193.

Charlton and Jehle,[1] working in the laboratory of St. Ann's Kinderhospital, Vienna, found, in apparently normal stools, B. paradysenteriae, Flexner-Manila type, present in two cases out of ten examined. These cases gave a negative history as to dysentery and diarrhea. The blood of the cases did not agglutinate the cultures obtained from the stools or the Flexner-Manila bacillus.

The following report comprises a study of 78 cases, of which 57 were children with normal stools and 21 were children suffering from acute or subacute diarrhea.

The first series of cases was undertaken during the months of March and April, 1903. At this time, a mild epidemic of dysentery existed in some of the wards at the Nursery and Child's Hospital, New York City. From the stools of several of these cases B. paradysenteriae, Park-Mt. Desert type, was isolated. In one case the organism was obtained from the stools before death and at autopsy from the mucous membrane of the lower colon. Thirteen infants having normal stools were selected from these infected wards for examination. In every instance the result was negative.

No special method was used to obtain the material for examination, plates being made as early as possible from the stools as normally passed.

During the month of August of the same year, coincident with the existence of dysentery in some of the wards, the study of normal stools was resumed. On this occasion the cases were taken from the reception ward where dysentery was, presumably, not present. The children examined were for the most part nurselings.

Two cases had had dysentery a few weeks previous to this time; from one case B. paradysenteriae had been isolated. At the time of the examination, both infants had recovered and the stools were normal. In neither case was B. paradysenteriae found. Seven of the remaining ten cases were also negative, while the tenth case was positive, giving the following history:

Case 1.—Age 24 months, nurseling, healthy. Had been in the reception ward three weeks; during this time the stools were normal and there was

[1] *Trans. Assn. American Phys.*, 1904, 19, p. 405.

no history of diarrhea before entering the hospital. The child developed pneumonia three days after the stools were examined and the organism found. In the beginning of the attack there was a slight amount of mucus, but in every other respect the stools were normal and remained so throughout the course of the disease. The blood gave a good reaction in dilution of 1:10 with the Flexner-Manila organism.

The organism isolated in this case in the proportion of one out of one hundred colonies possesses the following characteristics: Non-motile bacillus, ferments mannite and maltose without production of gas, does not produce gas on glucose or lactose, produces indol and nitrites, renders litmus milk slightly acid which later becomes alkaline. In the above features it corresponds to the Flexner-Manila type. In its agglutination reactions it resembles the Coney Island and Salant organisms which were isolated from cases of typical clinical dysentery.

A polyvalent dysenteric horse serum agglutinates this bacillus completely in the dilution of 1:500. It raises specific agglutinins in the animal body for itself and common agglutinins for other mannite fermenting types of the dysentery organism. The following table shows by the absorption tests its relation to the Coney Island and Salant cultures:

TABLE 1.

SERUM OF A HORSE INOCULATED WITH SHIGA, FLEXNER-MANILA, AND PARK-MT. DESERT TYPES OF B. DYSENTERIAE EXHAUSTED WITH THE CONEY ISLAND AND MT. DESERT TYPES.

Cultures	Serum before Exhaustion						Serum after Exhaustion with Coney Island Type						Serum after Exhaustion with Mt. Desert Type					
	20	50	100	200	500	1000	20	50	100	200	500	1000	20	50	100	200	500	1000
Japan—Shiga	++	++	++	++	++	++	++	++	++	++	++	−	++	++	++	++		
Manila—Flexner	++	++	++	++	++	++	++	++	++	++	++		++	++	++	++		
Mt. Desert—Park	−+	++	++	++	++	++	++	1					−	−				
Coney Island—Collins	+−	++	++	++			−						++	++	−			
New York—Salant	++	++	++	++			−						++	++	−			
Normal—Collins	++	++	++	++			−						++	++	−			

++ Complete agglutination, 1 tendency, − negative.

In the above table the three last cultures exhibit a similar reaction after exhaustion. It is of interest to note that the production of common agglutinins for the Coney Island type is much less than for the Mt. Desert type.

In the above series of examinations the material was obtained by irrigation of the lower colon with sterile water. Plates were made from the first and last parts of the washing. From the

latter, bits of mucus could readily be picked out. After withdrawal of the tube, the rectum was swabbed with sterile cotton and plates were made also from the swab.

The next study of 54 cases, obtained from several different sources, was made during the months of July, August, and September, 1904. Thirty-three of these cases were children with normal stools, and 21 were children suffering from acute or subacute diarrhea. In obtaining material for examination in these cases, I used the method devised by Dr. William H. Park. This consists of a blind glass tube with quite a good sized opening on the side. The tube is inserted into the rectum and the mucous surface, exposed through the opening in the side, is washed with sterile water. A cotton swab is then passed several times over the surface of the membrane; the swab is placed in a tube of broth and plates are made from this. A platinum loop was tried for scraping the mucous membrane, but it offered no advantage over the swab and was more irritating. In some cases irrigation of the lower colon supplemented the use of the tube, but as the use of the latter eliminated, to a very great extent, the gas producing organisms, it proved a more satisfactory method.

Of the normal cases examined, 17 were obtained through the courtesy of Dr. Reed from the Babies' Hospital, New York City. These were bottle fed babies ranging in age from three weeks to 21 months. Dysentery existed in the wards at this time, and some gave a past history of diarrhea, but, at the time of the examination, the stools were normal. In none of these 17 cases was B. dysenteriae or paradysenteriae found to be present.

Thirteen normal cases taken from the Foundling Hospital, New York City, were children ranging from two and a half to seven years of age, who had been in the wards where dysentery existed from three weeks to three years. Out of the 13 cases two were positive, the history being as follows:

Case 2.—Age 7 years. Eczema. Had been in the ward three years, and during that time there was no history of diarrhea. The stools were normal at the time of examination. The blood of the child reacted fairly in dilution of 1:20 with the Mt. Desert type of organism, but failed to show even a trace with the Shiga or Flexner-Manila type.

From the mucous surface of the rectum B. paradysenteriae, Mt. Desert type, was isolated. This organism agglutinated with a polyvalent dysenteric horse serum in the dilution of 1:500. With the Mt. Desert specific serum,* it agglutinated in the dilution of 1:200, which was the full agglutinating index of the serum, but failed to agglutinate with the Flexner-Manila or Shiga specific serum.

Case 3.—Age 3 years. Chronic conjunctivitis. One year in the ward, during which time there was no history of diarrhea. The stools were normal at the time. The blood in this case reacted feebly in dilution of 1:20 with the Flexner-Manila organism and not at all with the other types.

From the rectal mucous membrane B. paradysenteriae, Flexner-Manila type, was isolated in proportion of 1:50 of the colonies fished. It agglutinated with the polyvalent serum in the dilution of 1:500; with Flexner-Manila specific 1:100, and was negative with the specific sera of the other types.

During the past summer an epidemic of dysentery occurred in several districts, widely separated from each other, on Staten Island. Through the kindness of Dr. Walser and Dr. Patterson of Staten Island, the blood and stools from several of the dysenteric patients were obtained for examination and, as far as observed, only the Shiga type of organism prevailed.

From one of these infected neighborhoods where the sanitary surroundings were very poor three normal cases were taken under the following conditions:

Case A.—Age 7 years. No history of diarrhea. Three members of the family had had dysentery of the Shiga type. One case, in a child of three years, existed at the time of examination of the normal child, one adult case occurred three weeks previous, and one case two weeks later. Examination of the stools of the normal case was negative.

Cases B and C were taken from two children, two and five years of age, living directly oppposite to Case A. One child in this family, three years of age, had dysentery, Shiga type, at the time. The stools of the two normal children were negative.

The 21 cases of acute and subacute diarrhea were derived from the Babies' Hospital and the dispensaries of Bellevue Hospital and the German Polyclinic. The infants were bottle

*By specific serum is meant a polyvalent dysenteric serum from which all agglutinins have been absorbed except those for the one type.

fed and were from six to eighteen months of age. The acute cases presented, in general, the following symptoms:

The onset was sudden, with vomiting, rise of temperature, which was generally high, stools frequent, thin and watery in appearance and sometimes green and frothy. The prostration was marked and mortality high.

In the subacute cases the vomiting was absent, the rise of temperature moderate, if any, the stools thin and sometimes green, with some mucus but no blood; the emaciation was great.

In these cases, the contents of the colon were examined as well as the mucous surface of the rectum, and in no instance was B. dysenteriae found.

Charlton and Jehle[1] did not find the dysentery bacillus in the stools of children suffering from so called summer diarrhea, and Weaver[2] also failed to demonstrate it as the cause of the summer diarrhea of children.

The limited number of cases reported in which B. dysenteriae has been found in the stools of healthy individuals, or those suffering from disease other than dysentery, together with the fact that thus far the organism has not been found normally in adults, renders the interpretation of this phase of the subject difficult.

Flexner[3] has pointed out the advisability of questioning the nature of this organism as to its purely parasitic existence. Investigations along this line will aid greatly in determining the significance of the presence of the organism in the normal individual and in cases where the definite clinical symptoms of dysentery are absent.

Reasoning from analogy, we would readily assume that B. dysenteriae, like B. diphtheriae and the pneumococcus, may lead a harmless existence in the normal individual until a lowered vitality of the tissues invaded offers a suitable soil for the full development and elaboration of its toxines.

Wollstein[4] calls attention to the difficulty of obtaining reliable histories in the cases of children. This seems to be the experience generally of those who have to do with children. Very few

[1] *Loc. cit.* [2] *Jour. Inf. Dis.*, 1905, 2, p. 81. [3] *Loc. cit.* [4] *Loc. cit.*

infants, especially of the class generally found in dispensaries and hospitals, escape diarrhea in some form in the early months of life. Hence the finding of the organism under such conditions should not lead us into drawing too definite conclusions.

The failure to find B. dysenteriae or paradysenteriae in infants suffering from so called acute and subacute summer diarrhea, even though the symptoms were severe and repeated examinations were made, would lead us to suspect some cause or combination of causes other than this organism as the etiological factor in these conditions.

I desire to express my gratitude to Dr. William H. Park, Director of the Research Laboratory of the Department of Health, New York City, and to Dr. Simon Flexner, Director of The Rockefeller Institute for Medical Research, for their suggestions and criticisms of this work.

NOTES ON THE COMMON MOSQUITOES OF BIHÉ AND BAILUNDO DISTRICTS, PORTUGUESE WEST AFRICA.*†

FREDERICK CREIGHTON WELLMAN.

THE following notes are based on the collection of *Culicidae* made by the writer in the region occupied by the districts of Bihé and Bailundo, Portuguese West Africa. The collection was begun in 1903, and most of the different species included in it were kindly determined by Mr. F. V. Theobald, of the South-Eastern Agricultural College, England, who is also describing those specimens which are new to science. None of the mosquitoes in this list (with one exception noted in its place) have been reported by other observers from the districts named, and the collection under consideration is the only one which, to the writer's knowledge, has ever been made of the *Culicidae* of this region. The mosquitoes are described in the chronological order in which they stand in the collecting-record. The writer desires to express here his sincere thanks to Mr. Theobald, and also to Mr. Austen, of the Zoölogical Department of the British Museum, whose interest in the distribution and habits of African blood-sucking diptera is well known, and with whom some correspondence regarding the subject of these papers has been carried on.

Culex viridis, Theob.

Theobald. (In letter from Mr. Austen.)

This green, sylvan, "garden" species cannot be mistaken for any other mosquito. I find, however, that it is occasionally confused with a green midge (*Chironomus pulcher*, Wied.) in company with which it is sometimes found. I have been unable to discover where this mosquito breeds, but I suspect that it may be in the small collections of water which are held in the leaves and bracts of large plants during the rainy season. Appears November to December.

* Received for publication, October 23, 1905.

† Published under the auspices of the American Society of Tropical Medicine.

Myzomyia funesta, Giles.

Theob., *Monograph of the Culicidae*, 1901, 1, p. 178. Giles, *Gnats or Mosquitoes*, 2d ed., 1902, p. 318.

The small size, position of the cross veins, costa with the apex pale, and three pale spots smaller than the intervening basal dark portions, and characteristic wing fringe with pale areas at the end of all the veins except the sixth, together with the banded, white-tipped palpi, black tarsi, thorax, and abdomen, sufficiently mark this mosquito. *M. rhodesiensis* is strikingly like this species, but can be distinguished from it by its unspotted wing fringe and black-tipped palpi.

FIG. 1.—*Myzomyia funesta*, Giles. ♀

M. funesta breeds in springs and clear, slow-running water. Very often found in houses. Does not offer to bite much in evening, unless one quietly sits or stands away from the lamp. This mosquito is expert in crawling into small places and often gets under a mosquito curtain. A very common anopheline,[1] and the principal carrier of malaria in this region.

Cellia pharoensis, Theob.

Mono. Culicid., 1901, 1, p, 169. *Gnats or Mosquitoes*, 1902, p. 302.

The characteristic dark gray thorax with two black, eyelike spots is well shown. The tufts at the sides of the abdominal segments, the scaled antennæ, and the large, white spot in front of the transverse wing veins render the species unmistakable. In addition to the fact of its being an anopheline, this mosquito and the following one have a special

FIG. 2.—Wing of *Cellia pharoensis*, Theob. (After Giles.)

[1] *Jour. Trop. Med.*, 1904, 7, p. 53.

interest in the light of Low's statement that at least one species of *Cellia* can act as host for *F. nocturna*.

Cellia squamosa, Theob.

Mono. Culicid., 1, p. 167. *Gnats or Mosquitoes*, p. 314.

The somewhat different wing markings and the three conspicuous white longitudinal stripes on the pleuræ easily separate this from the foregoing species. The coal black, densely scaled abdomen with its projecting tufts on the posterior borders of the segments make the mosquito a striking object. It is a rather bold anopheline, and comes readily to a lighted lamp in the evening. Most abundant from November to February.

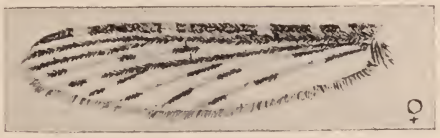

FIG. 3.—Wing of *Cellia squamosa*, Theob.

Anopheles wellcomei, Theob.

Theob. (In letter from Mr. Austen.)

The remarkable size, broad wing markings, and white hind "feet" of this species make it easy to pick out, even without detailed examination of its micro-characteristics. It is generally found near large streams, and I believe that it breeds in running water. A pertinacious biter. Bold even in day time. I find the following note in my diary for June 12, 1905: "Bitten in bright sunlight on the banks of the Cutato River by an *A. wellcomei*, Theob." In shade it will follow one for some distance.

Pyretophorus austeni, Theob.

Entomologist, 1905, 38, p. 102.

This is a new species described from this region. It is a dark mosquito resembling *P. costalis*, Loew, for which I first mistook it,[1] but differs from the type in several particulars, among which the most prominent are the characteristic thoracic ornamentation and wing markings. The dark thorax is clothed with broad,

[1] *Jour. Trop. Med., loc. cit.*

curved, snowy-white scales. Costa with two white spots and a minute third spot near the base. Length, five mm. It is a quiet and unobtrusive, but domestic, mosquito which will breed in fairly muddy pools. It is of interest to point out, in mentioning this mosquito, that the type of the genus *P. costalis* has been proved to carry malaria, and is said to be an intermediate host of *F. bancrofti*. Most abundant in December to March.

Danielsia wellmani, Theob.

Entomologist, 1905, 38, p. 103.

Another new species from this region which Mr. Theobald has done me the honor to name for me. It is a beautiful and distinct species the chief characters of which are its thoracic and abdominal markings and leg banding. The thorax is black with narrow, curved, bronze scales, but with a cream-colored scaled area on either side which expands and meets anteriorly. Abdomen, violet-black with basal, white, lateral spots, becoming median on the last two segments. Legs, dark brown, mid and hind pair with broad, basal, white band to the metatarsi and first tarsi. The description states that the scutellar scales are somewhat broader than in the type of the genus (*D. albolineata*). Length four mm. The ova are brownish and the larvæ are characteristically marked. I hope to publish a study of the life-history at some future time.

FIG. 4.—Head of larva of *D. wellmani*, Theob. (Magnified.)

Culex taeniorhynchoides, Giles.

Jour. Trop. Med., 1904, 7, p. 369.

A new species. The wings are dark and unspotted. Thorax sooty with golden, curved scales and with dense tufts of black scales over the roots of the wings. The black proboscis has a definite ocherous band on the middle. It is a rather large species. Described from a specimen taken in Bihé district, and sent by Dr. A. Y. Massey of Toronto, Canada.

Myzorhynchus umbrosus, Theob.

Mono. Culicid., 1901, 3, p. 89.

This mosquito, determined by Colonel Giles, presents no striking differences from *Anopheles barbirostris*, Wulp[1] or *Mzr. pseudo-barbirostris*, Ludlow.[2] It has, however, but a single costal spot just inside the apex and a very pronounced yellow and black apical fringe. It seems to be a sylvan species. Length five mm.

Mansonia africana, Theob.

Mono. Culicid., 1901, 1, p. 276. *Gnats or Mosquitoes*, 1902, p. 357.

Mansoniae are perhaps the easiest of all the Culicines to determine. Their peculiar brindled appearance and the densely scaled wings with broad, asymmetrical, flat (bracket-shaped) scales on both sides of the veins render them unmistakable. The only genus which they resemble is *Mucidus*, but they lack its characteristic rough "moldy" appearance, and they possess scales of a somewhat different shape.

FIG. 5.—Wing scales of (A) *Mansonia* and (B) *Mucidus*, to show difference in shapes. (After Theobald.)

M. africana is distinguished by its thorax, which is ornamented by broad lines of golden-brown and silvery scales and two pale spots. The legs are curiously mottled and banded. Length about four mm. Probably a spreader of filariasis in some regions.

[1] Leyden Museum *Notes*, 6, p. 48. (Quoted in *Gnats or Mosquitoes*, 1902, p. 308.)

[2] *Jour. N. Y. Entom. Soc.*, 1902, 10, p. 127.

(*To be continued.*)

CORRIGENDA.

P. 270, line 2: instead of "Scolephagus," read "Scolecophagus."
P. 308, under Fig. 4, Plate 8: "Magnification about 985."
P. 311, line 15: instead of "diptheria," read "diphtheria."

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The
Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. II *November 25, 1905* No. 4

PUBLICATIONS FROM THE NEW YORK STATE CANCER LABORATORY. NO. 30.*
H. R. Gaylord, G. H. A. Clowes, G. N. Calkins.

SOME ARTEFACTS IN MOUSE CARCINOMA.

G. N. CALKINS AND G. H. A. CLOWES.

THE present paper deals with certain cell inclusions in mouse carcinoma, inclusions due to technical errors, but offering some evidence of an interesting type of intracellular necrosis.

The history of cell inclusions in carcinoma clearly shows that products of necrosis have been occasionally mistaken for parasites of one kind or another, and the possibility—especially after fixation with any mixture containing salts of mercury—of similarly misŧaking artefacts is apparent to all laboratory workers.

The difficulty in removing precipitates from Zenker-fixed material is generally recognized, and treatment in bulk with iodized alcohol (often for weeks), is advised in most laboratory manuals, and for large pieces, subsequent treatment of paraffin sections is recommended. Rawitz's *Leitfaden für histologische Untersuchungen* advises treatment of pieces fixed in Zenker with "70 per cent alcohol with enough tincture of iodine added to give it a port wine color, the iodine and alcohol to be changed until the color no longer disappears" (1895, p. 17). Mercier[1] recommends 10–15 days at least in iodized 90 per cent alcohol and advises the use of clear tincture of iodine or strong concentrated iodized alcohol.

* Received for publication July 20, 1905.

[1] *Zeit. für w. Mik.*, 1894, 11, p. 471.

The inclusions that we have to describe are not affected perceptibly by such treatment. The usual treatment of material in this laboratory is the same as that recommended in the majority of laboratory manuals, and the usual "mercury" deposits are frequently seen and recognized as such. In the series of mouse experiments with which the State Cancer Laboratory has been occupied for more than a year, such inclusions have been seen in the cancer tissues and passed over without much thought. In one case, however, the tumor when partially grown was stirred up thoroughly with a needle and then left for further developments. After some days it was observed that the tumor had grown smaller and the mouse showed signs of spontaneous recovery. The tumor was then removed and hardened in Zenker's fluid, treated as usual with iodized alcohol, embedded in paraffin, sectioned and stained according to the usual methods. The most wonderful variety of crystals and deposits, both intra and intercellular, were found in these sections, some comparatively large, others exquisitely minute.

The most common type of crystalline deposit in this tumor is comparatively small, dense, and nearly homogeneous, and with clear evidence of radiate structure usually with an eccentric focus. In other forms the radii are more marked, clearly defined, and the entire structure is fan-shaped (Plate 17, Figs. 1 and 4). In still other cases this fan structure is elaborated in much greater detail and many of the radii are carried out beyond the periphery as elongate, filamentous processes (Plate 18, Fig. 1). The ends of these processes bear local, cylindrical or sausage-shaped thickenings which give to the structure a striking resemblance to a fungus-growth.

In other cases of intercellular inclusions the crystallization does not result in uniform, definite masses, but in filaments without the dense basal portion. Such structures may start from the periphery of a hypertrophied cell, in which irregular masses of the deposit and the degenerated nucleus alone remain; while at the ends of the filaments again, are the enlarged, clublike, cylindrical masses (Plate 18, Fig. 2).

In still other cases the type of the crystallization differs from

both of the above, resembling more than anything else the plan of structure of hoar frost on glass. Minute crystalline masses are attached end to end and a running crystalline structure results which may extend over an area of at least one-half a square millimeter.

The intracellular and intranuclear inclusions are even more remarkable than the intercellular. For convenience they may be distinguished as the "cytoplasmic" and the "nuclear" forms. The cytoplasmic forms are always in vacuoles which vary in size from minute vesicles to great intracellular spaces considerably larger than the nucleus. These small vesicles are seen in the vicinity of the nucleus and usually in the minute depression formed by the crescent-shaped nucleus (Plate 18, Figs. 6–10). Within the vacuoles are many curious, separate, filiform structures with characteristic spherical swellings at the ends (Plate 17, Figs. 5 and 6; Plate 18, Figs. 9 and 10). These filaments are usually single and separate, but in some cases they form a botryoidal group of characteristic arrangement (Plate 18, Fig. 8). Occasionally there is a central mass with irregular radiating branches (Plate 18, Fig. 13), while in a few cases the radiating structure, central mass, and knobbed radii furnish an intracellular parallel to the larger intercellular fan-shaped structures (Plate 18, Fig. 12).

Even more remarkable are the intranuclear filaments and knobs. Inside the nucleus there are minute masses similar in size and shape to the swollen ends of the radiating filaments (Plate 18, Fig. 5); or larger, swollen masses with irregular protuberances of variable length (Plate 18, Figs. 4 and 5), or finally, there may be long filaments with knobs on the ends similar to the knobbed filaments of the cytoplasmic forms (Plate 18, Fig. 3; Plate 17, Fig 3).

Attempts to stain these various structures are not successful; in some cases the structures have a faint homogeneous color which cannot be interpreted as a true staining reaction. The filaments and knobs are never stained even as much as this, although blackened by treatment with ammonium sulphide. Usually they present the same glassy or glairy appearance that characterizes fat under the microscope, and this appearance is more definite in the

terminal swellings than in the substance of the filaments or the rays of the fan structure.

Unfortunately, on account of the limited amount of material at our disposal, it was impossible to make a thorough chemical investigation of these products. From micro-chemical tests however, it was found:

That a prolonged treatment with cold Lugol's solution removes all the bodies referred to above, a slight, amorphous residue being left within a few of the vacuoles.

That a short treatment with Lugol's solution leads to partial removal of the crystals, especially the large, extracellular, fan-shaped variety.

That treatment of the sections with dilute sulphuretted hydrogen or ammonium sulphide leads to a distinct blackening, the intensity of which is dependent upon the length of time that the sections are exposed to the reagent and the concentration of the latter. Sections so treated, when subsequently exposed to Lugol's solution at 50°, are entirely cleared in the course of half an hour.

These tests warrant the conclusion that the semi-crystalline compounds consist, at least in part, of mercury salts derived from the hardening agent. It should however be noted that whilst the large fan-shaped bodies blacken intensely under the influence of ammonium sulphide. the smaller intracellular and intranuclear bodies are blackened to a less marked extent and the fine filaments to which knobs are attached (Plate 18, Figs. 6–10), are apparently influenced very slightly, if at all.

The solubility of the various types of inclusions was tested in a series of organic solvents. Alcohol exerts very little influence in the cold, but at 50° for half an hour sections are entirely cleared of all inclusions, merely a slight residue being observed in some of the vacuoles. Ether, acetone, and admixtures of alcohol, ether, and acetone exert a similar, though possibly less marked, solvent action than does alcohol alone. Petroleum-ether and xylol produce very little effect, even at 50°. Chloroform apparently exerts more action than the latter and less than the former group of reagents. These results, when taken with the fact that salts of mercury are far more soluble at high tempera-

tures in organic solvents than at normal temperatures, further justify the conclusion that the structures described above consist, at least in part, of salts of mercury.

To summarize the above results of chemical tests it may be said that mercury is unquestionably present in larger or smaller quantities in all the inclusions, both intra and intercellular. From the behavior of the crystals, especially the intracellular and intranuclear varieties, toward ammonium sulphide and other reagents it appears possible that the mercury salt is either deposited upon some product of necrosis of a fatty nature, or intimately associated with the latter in the form of a chemical compound. It has been found impossible to reproduce such forms from normal tissue or by means of a hardening agent admixed with various known products of decomposition. If any peculiar products of necrosis are associated with the salts of mercury in the inclusions referred to, the readiness with which they are removed by hot organic solvents and their behavior toward Lugol's solution and other reagents, as well as their lack of any characteristic micro-chemical reactions would lead us to suppose that they are of a fatty nature. In any case, it must be said that the figures observed resemble very closely the various forms assumed by fatty acids and fatty acid compounds when admixed with impurities.

While these deposits are to be interpreted as artefacts, the fact must not be overlooked that something of an unusual nature is present in these cancer cells and tissues, upon which the salts of the fixing agent work in forming the deposits of various kinds. The morphological evidence supports the view that necrosis of a marked type is in progress in these cancer cells; a necrosis beginning with cytoplasmic degeneration in the immediate vicinity of the nucleus and ending in great, hypertrophied and highly vacuolated, degenerated cells. The earliest morphological evidence of such degeneration is a minute vacuole in an otherwise normal cell (Plate 18, Fig. 6). It is conceivable that the material of this vacuolated region is metamorphosed into a substance upon which the salts of the Zenker fixing agent act to form the curious knobbed filaments. The necrotic cytoplasmic region

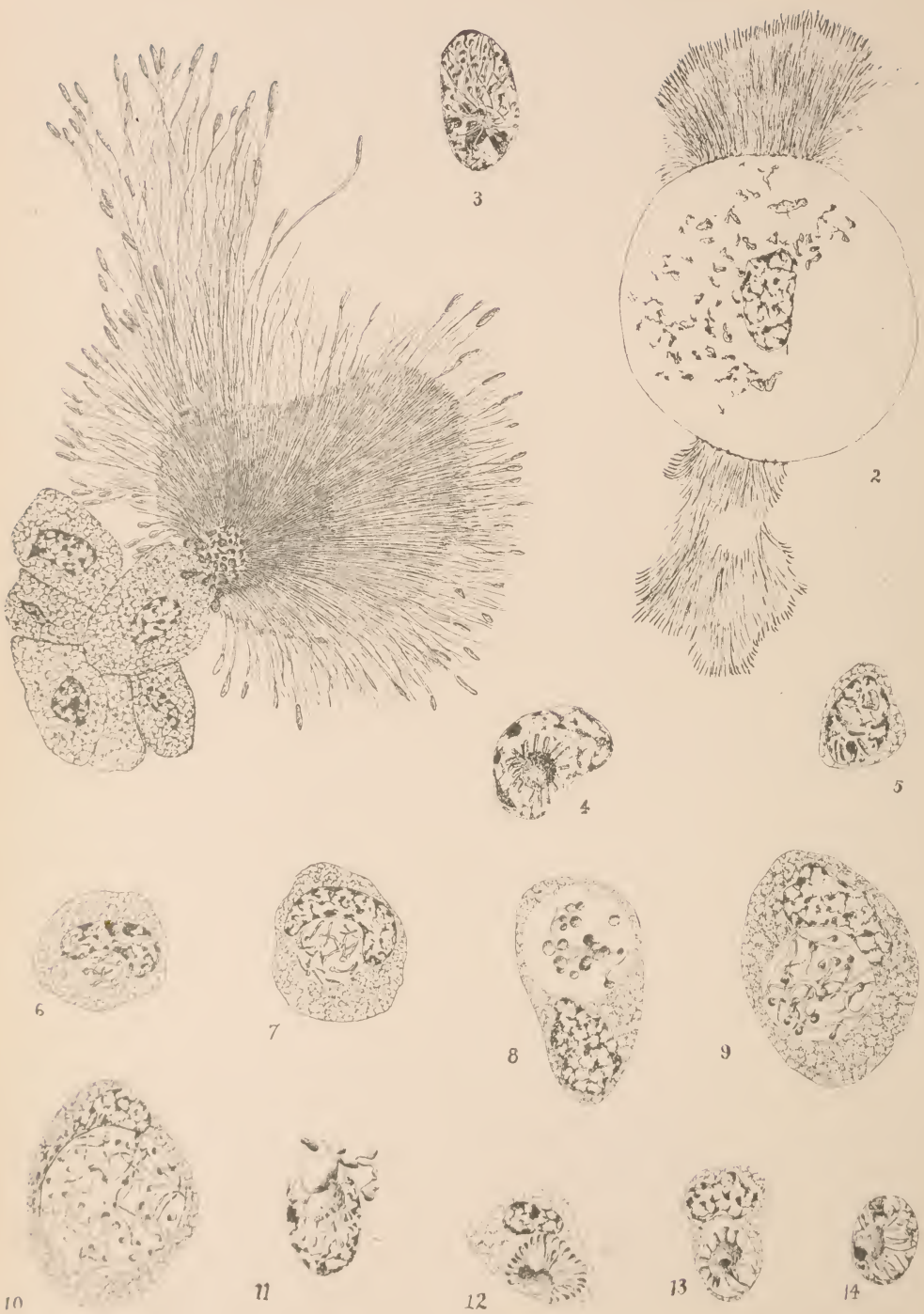
increases in volume with further degeneration through continued action of the disease, and the fixing material acting on this larger mass produces an increased number of knobbed filaments in a greater cytoplasmic vacuole (Plate 18, Figs. 7–10). The ultimate result of this degenerating change is a huge cell skeleton consisting of a well filled out cell membrane, degenerated nucleus, and irregularly distributed material where the cytoplasm had been. The fixing agent, acting on this stage of necrosis, gives few or none of the intracellular knobbed filaments, but, radiating out from the membrane into the surrounding intercellular spaces, may be found masses of such filaments with the characteristic terminal knobs (Plate 18, Fig. 2). From such evidence it might be inferred that the necrotic material produced in the degenerating cell had exuded through the cell membrane and was in the course of a general distribution when combined with the salts of the fixing agent. In still other regions the direct connection between the degenerated cell and the deposits is not so close, but throughout the immediate environment may be found the characteristic crystalline aggregates described above as a "running crystalline structure."

If our line of reasoning is correct, it appears that we have in this method of treating cancer tissues, i. e., by the creation of artefacts, a rough means of following morphologically the course of this particular type of degenerative change and the distribution of its products.

From a purely technical point of view, on the other hand, these results point out, in the clearest manner, the need of extreme caution in dealing with Zenker-fixed material. The ordinary precautions advised in every laboratory guide-book are inadequate, and more drastic measures must be employed to clear the tissues and cells of foreign bodies. For this we recommend treatment of paraffin sections for at least 30 minutes with Lugol's solution (potassium iodide plus iodine in water), or treatment of paraffin sections for about two hours with alcohol at a temperature of 50°.

Buffalo, June 1905.

PLATE 18.



C. N. Calkins, del.

SOME ARTEFACTS in MOUSE CARCINOMA

PLATE 17.

FIG. 1.

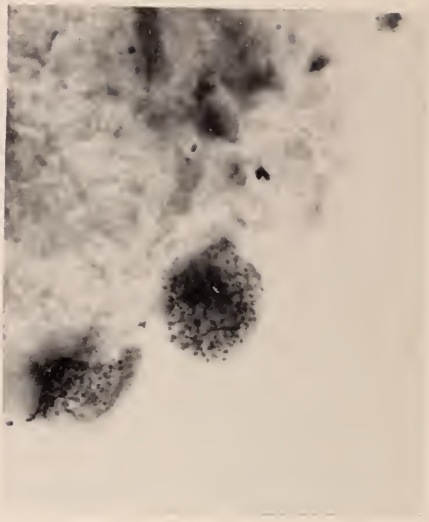


FIG. 2.

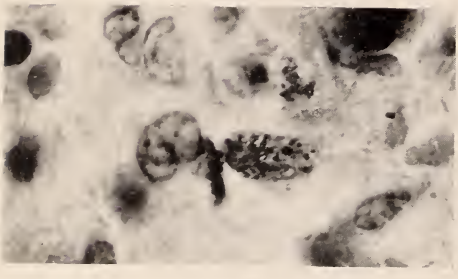


FIG. 3.

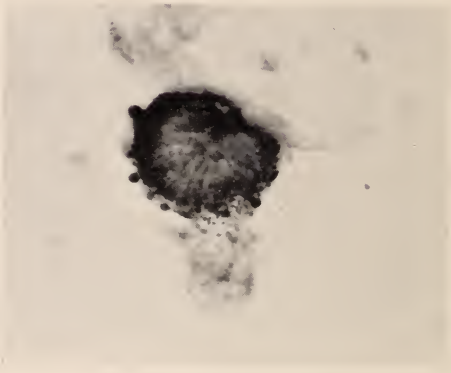


FIG. 4.

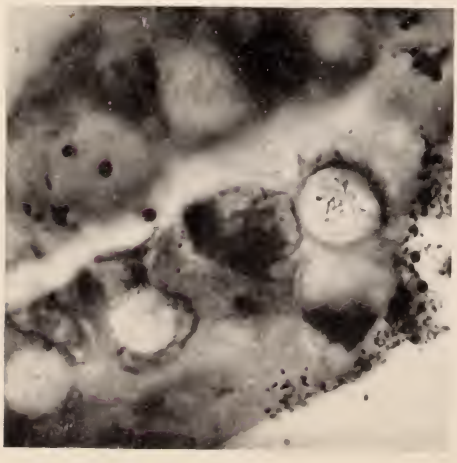


FIG. 5.

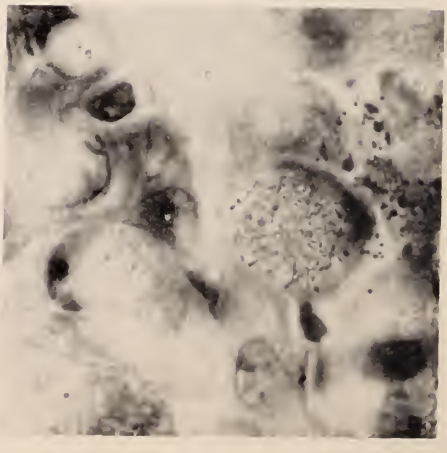


FIG. 6.

DESCRIPTION OF PLATES.

PLATE 17.

(Microphotographs × 1000.)

FIGS. 1 and 4.—Microphotograph of small and large intercellular crystalline deposits, with radiating structure and granules of mercury.

FIG. 2.—Microphotograph of similar inclusion without radiate structure.

FIG. 3.—Microphotograph of nucleus with inclusions (Cf. Plate 18, Fig. 3).

FIG. 5.—Microphotograph of smaller vacuoles and crescentic nuclei.

FIG. 6.—Microphotograph of very large intracellular vacuole, crescentic nucleus and intra-vacuolar deposits.

PLATE 18.

(Camera lucida drawings from permanent preparations.)

FIG. 1.—Large intercellular crystalline deposit with radiate structure; the rays extending beyond the periphery are provided with terminal thickenings. × 1800.

FIG. 2.—Degenerate cell with remains of nucleus and flocculent deposit in place of the cytoplasm. From the cell periphery radiating fibers with terminal thickenings reach out into surrounding spaces. × 1000.

FIG. 3.—Nucleus with intranuclear deposits in fiber-form and with terminal knobs. × 1800.

FIG. 4.—Intranuclear deposit. × 1800.

FIG. 5.—Intranuclear deposits of variable size. × 1800.

FIG. 6.—Epithelial cell with beginning of vacuolar degeneration in the depression formed by the crescentic nucleus, and with intravacuolar inclusions. × 1800.

FIGS. 7, 8, 9, and 10.—Epithelial cells with vacuoles of increasing size and with accumulating, characteristic. knobbed inclusions. (Cf. Plate 17, Figs. 5 and 6.) × 1800.

FIGS. 11, 12, 13, and 14, various forms of intracellular and intranuclear deposits. × 1800.

THE ANTAGONISM OF BACTERIA AND THEIR PRODUCTS TO OTHER BACTERIA.*†

LEO F. RETTGER.

(*From the Sheffield Laboratory of Bacteriology, Yale University.*)

EVERYWHERE in nature we see numerous examples of two opposing biological activities. On the one hand, living organisms frequently derive mutual benefit by association; on the other, the presence of one organism, or its products, is inimical to the life and growth of certain other forms. These principles are not limited to animals and the higher forms of plant life, but occur also as low down in the scale as bacteria themselves. Nor do we wonder at this when we consider how numerous and unlike are the products of different bacteria.

Recent investigations show that certain types of bacteria thrive best when associated with each other; for example, the typhoid bacillus and streptococcus.[1] Again, there are numerous instances in which bacteria exert antagonistic action toward each other. It is with the latter theme that this paper will deal.

Lode[2] claims for a certain diplococcus, whose identity he was unable to establish, that it possessed antagonistic properties toward a number of well known bacteria; among them, B. anthracis, Staphylococcus pyogenes aureus, the bacillus of fowl cholera, the typhoid bacillus, and the cholera vibrio. In culture tubes and plates this diplococcus tended not only to check the growth of the above mentioned organisms, but also to kill them. Furthermore, culture fluids retained this property after filtration through a Berkefeld filter. Animal experimentation gave negative results, however.

According to Turro,[3] subcutaneous injections of beer yeast protect a rabbit against fatal experimental streptococcus and

* Read before the Society of American Bacteriologists at Philadelphia, December 1904.

† Received for publication July 10, 1905.

[1] VINCENT: *Ann. de l'Inst. Pasteur*, 1893, 7, p. 141; and *Compt. rend. Soc. de Biol.*, 1892, 44, p. 597.

[2] *Centralbl. f. Bakt.*, 1903, 33, p. 196.

[3] *Centralbl. f. Bakt.*, 1903, 34, p. 22.

staphylococcus infection. In fact, beer yeast has been employed as a remedy in cases of erysipelas and smallpox (the latter during the suppurative stage).

Much has been said and written about Emmerich and Löw's[1] "Pyocyanase-Immunproteïdine." A brief review of the work of these authors will not be out of place here. Emmerich and Löw showed that certain pathogenic organisms produce proteolytic enzymes both in artificial culture and in the animal body, which have the power of dissolving or digesting the bacteria that elaborate them; for example, the streptococcus of swine erysipelas and B. anthracis. Again, other microorganisms produce enzymes which are able to digest not only the organisms themselves, but possess this property in respect to other bacteria. The most interesting representative of this class is perhaps the pyocyaneus bacillus.[2]

Emmerich and Löw have named the active "bacteriolytic enzyme" of B. pyocyaneus, *pyocyanase*, and have separated it in a rather impure form from old broth cultures by filtration, concentration of the fluid to a small volume, dialyzation, and finally precipitation with alcohol and ether. Extremely small quantities of the pyocyanase prepared in the above manner destroyed millions of diphtheria, cholera, typhoid, and plague bacilli in but a few seconds of time, and rendered their poisonous products inactive. The pyocyanase showed marked antagonism also toward Streptococcus pyogenes, Staphylococcus pyogenes aureus and the anthrax bacillus.

Of special interest in the work of Emmerich and Löw is the application of the principle of bacterial antagonism to the protection of animals against fatal infection by the anthrax bacillus. At first varying quantities of pyocyanase (dissolved in water) were injected into rabbits at about the same time that they received a fatal dose of an anthrax culture. The results were far from satisfactory; and this the authors explained on the supposition that pyocyanase is not a very stable body, and that soon

[1] *Zeitschr. f. Hyg.*, 1899, 31, p. 1; *ibid.*, 1901, 36, p. 9. See also, Emmerich: *Centralbl. f. Bakt.*, 1902, 32, p. 821; Löw and Korschun: *ibid.*, 1902, 31, p. 1; Emmerich and Trommsdorff: *ibid.*, 1903, 33, p. 627.

[2] Blagovestchensky: *Ann. de l'Inst. Pasteur*, 1890, 4, p. 689.

after its introduction into an animal it is in a large measure destroyed by the animal tissues, before it can exert its protective function. It was conceived that if the pyocyanase could be converted into some more stable form, better results would be attained. Consequently, Emmerich and Löw prepared their so-called "Immunproteïdine" by mixing the pyocyanase with blood or other animal tissues, in this way presumably bringing about its combination with certain of the tissue proteids. With the "Immunproteïdine" they were able repeatedly to protect rabbits against fatal infection by anthrax. Although the work of Emmerich and Löw has received considerable criticism, and much remains to be desired from the practical standpoint, it has been substantiated, in a large measure at least, by a number of other investigators, particularly Vaerst,[1] Krause,[2] and Tavernari.[3]

Quite recently Dr. Maher,[4] of New Haven, published the results of some work carried on by him on the subject of bacterial antagonism. He claims to have repeatedly cured tuberculosis in man by subcutaneous injections of a certain spore-bearing bacillus which he isolated originally from sour milk—in all probability B. mycoides. Kinghorn[5] employed this same bacillus in connection with experimental tuberculosis in rabbits, but obtained negative results.

For some time I have been engaged in a study of the chemical and physiological properties of B. prodigiosus, more particularly its products. Among other things, I soon found that dead prodigiosus bacilli and their products had a distinctly protective action against infection by the anthrax bacillus. Subcutaneous or peritoneal injections of prepared "prodigiosus powder," which were made at the same time and at certain intervals after inoculation with an ordinarily fatal dose of anthrax bacilli, protracted the life of the guinea-pigs from 14 to 72 hours, and in one instance entirely prevented the death of the animal. The method of investigation was as follows:

[1] *Centralbl. f. Bakt.*, 1902, 31, p. 293. [2] *Ibid.*, p. 673. [3] *Ibid.*, p. 786.

[4] *New York Med. Jour. and Phila. Med. Jour.*, 1904, 79, p. 163.

[5] *Jour. of Med. Res.*, 1904, 10, p. 249.

The prodigiosus powder was prepared from potato growths, which were four to five days old, by scraping the growths with a sterile section lifter, thoroughly macerating in a mortar under chloroform at certain intervals covering a period of 24 hours, and completely drying in an exhaust desiccator. Finally, the dried mass was ground to a fine powder. The entire process was carried on under aseptic conditions.

A prodigiosus powder prepared in the above manner possesses the following properties: Its color varies from a pink to a deep red. It is sterile. It coagulates milk within a few hours, and redissolves the coagulum. It rapidly liquefies gelatin, and has a marked proteolytic action in general. It inverts cane sugar. Finally, it possesses pronounced toxic action when injected into guinea-pigs and rabbits. The chief effects of its toxic action are fever, soreness of the eyes, loss of appetite, emaciation, and frequently diarrhea. Marked necrosis usually occurs at the point of injection.

Since the powder possessed such toxic properties, it was necessary to inject very small quantities. For this purpose definite amounts (usually 0.05 to 0.1 gram) of the sterile powder were mixed in a test tube with 10 c.c. physiological salt solution. After filtration through loosely packed absorbent cotton, one or two cubic centimeter portions (5 to 20 mg.) were injected by means of a hypodermic syringe. The injections were usually made near the site of the anthrax inoculation. Owing to the minute quantities of powder employed, the injections were, as a rule, repeated several times at more or less regular intervals.

The anthrax material was prepared as follows: A "standard" platinum loopful of a 24 hour agar growth of the anthrax bacillus was thoroughly mixed with 10 c.c. physiological salt solution (sterile). One cubic centimeter of this suspension was introduced into a second tube (10 c.c. salt solution); and from this again one cubic centimeter was brought into a third tube. Definite portions of the third and last dilution were injected into the animals by means of a syringe. Bacterial counts were made by the usual method, and in this way the number of bacilli injected was approximately ascertained.

EXPERIMENT I.

a. Guinea-pig, weight 452 grams. Subcutaneous injections of prodigiosus powder: Feb. 24, 3 P. M. (50 mg.); Feb. 25, 3 P. M. (10 mg.); Feb. 26, 9 A. M. (10 mg.). Inoculation with 10,000 anthrax bacilli (subcutaneous), Feb. 24, 3:30 P. M. Died in 60 hours.

b. Control pig, weight 450 grams. Similar inoculation with anthrax bacilli. Died in 36 hours.

Difference in length of life = 24 hours.

EXPERIMENT II.

a. Guinea-pig, weight 425 grams. Subcutaneous injections of prodigiosus powder: Mar. 1, 4 P. M. (10 mg.): Mar. 2, 10 A. M. (10 mg.); Mar. 3, 10 A. M. (10 mg.) and 3 P. M. (5 mg.); Mar. 4, 10 A. M. (5 mg.) and 5 P. M. (5 mg.). Inoculation with 9,000 anthrax bacilli (subcutaneous), Mar. 3, 10 A. M. Died in 50 hours.

b. Control pig, weight 475 grams. Died in 36 hours.

Difference = 14 hours.

EXPERIMENT III.

a. Guinea-pig, weight 600 grams. Subcutaneous injections of prodigiosus powder: Mar. 15, 11 A. M. (25 mg.) and 4 P. M. (10 mg.); Mar. 16, 10 A. M. (10 mg.) and 4 P. M. (10 mg.). Inoculation with 5,000 anthrax bacilli (subcutaneous), Mar. 15, 4 P. M. Died in 120 hours.

b. Control pig, weight 560 grams. Died in 48 hours.

Difference in length of life = 72 hours.

Organs of prodigiosus pig apparently normal and sterile.

Anthrax bacilli in large numbers in organs of control pig.

EXPERIMENT IV.

a. Guinea-pig, 400 grams. Subcutaneous injections of prodigiosus material: March 24, 11 A. M. (25 mg.); March 25, 10 A. M. (10 mg.). Inoculation with 800 anthrax bacilli (subcutaneous), March 24, 11 A. M. Died in 72 hours.

b. Control pig, 500 grams. Died in 46 hours.

Difference in length of life = 26 hours. Comparatively few anthrax bacilli in organs of prodigiosus pig.

EXPERIMENT V.

a. Guinea-pig, 575 grams. Subcutaneous injections of prodigiosus powder: April 14, 10 A. M. (30 mg.) and 5 P. M. (15 mg.); April 15, 5 P. M. (15 mg.); April 16, 12 M. (15 mg.). Inoculation with 150 anthrax bacilli (subcutaneous), April 14, 10 A. M. Died in 56 hours. No anthrax bacilli found in organs of dead pig. Death probably due to prodigiosus action.

b. Control pig, 600 grams. Lived.

EXPERIMENT VI.

a. Guinea-pig, 500 grams. Injections of prodigiosus powder: May 3, 10 A. M. (10 mg. subcut.); May 4, 10 A. M. (10 mg. subcut.); May 5, 9 A. M. (5 mg.

intraper.); 4 P. M. (5 mg. subcut.); May 6, 9 A. M. (5 mg. intraper.); 5 P. M. (5 mg. subcut.); May 7, 12 M. (5 mg. subcut.). Inoculation with 1200 anthrax bacilli (subcutaneous), May 3, 10 A. M. Died in 120 hours.

b. Control pig, 475 grams. Died in 48 hours.

Difference in length of life = 72 hours.

EXPERIMENT VII.

Both of the guinea-pigs lived after injection of 45 anthrax bacilli.

EXPERIMENT VIII.

a. Guinea-pig, 600 grams. Subcutaneous injection of prodigiosus powder, May 18, 12 M. (8 mg.). Intraperitoneal injections: May 18, 4 P. M. (5 mg.); May 19, 11 A. M. (5 mg.); May 19, 5 P. M. (3 mg.); May 20, 10 A. M. (4 mg.); May 21, 10 A. M. (5 mg.); May 22, 11 A. M. (5 mg.). Inoculation with 1600 anthrax bacilli, May 18, 12 M. Animal lived.

b. Control pig, 600 grams. Died in 47 hours after anthrax injection.

EXPERIMENT IX.

a. Guinea-pig, 510 grams. Injection of prodigiosus powder: June 22, 11 A. M. (5 mg. intraper.); 8:30 P. M. (4 mg. subcut.); June 23, 10 A. M. (5 mg. subcut.); 9 P. M. (5 mg. subcut.); June 24, 3 P. M. (5 mg. intraper.); June 25, 9 A. M. (3 mg. intraper.); 8 P. M. (2 mg. subcut.). Inoculation with 1000 anthrax bacilli (subcutaneous). Died in 90 hours. Comparatively few anthrax bacilli in organs of pig.

b. Control pig, 500 grams. Died in 65 hours.

Difference in length of life = 25 hours.

Let us briefly summarize the results of the nine experiments recorded here. In six of the experiments the life of the guinea-pigs was prolonged 14, 24, 25, 26, 72, and 72 hours respectively; while in one instance the animal quite recovered. It should be added, also, that in every case but one, microscopic examination revealed the anthrax bacilli to be far less numerous in the organs of the prodigiosus animals than in those of the control pigs. In fact, no bacilli could be found in one of the guinea-pigs (Exp. III). Here death was due, in all probability, to the toxic action of the prodigiosus material itself, and not to anthrax. In Experiment V it is likely, also, that the animal died in consequence of excessive dosage with the prodigiosus powder, as the organs proved to be sterile, and the control animal lived. In Experiment VII both animals lived after an injection of only 45 anthrax bacilli.

Besides the series of experiments recorded above, others were tried on rabbits and guinea-pigs. In three of these experiments, a prodigiosus powder was employed which was not sterile, and

the animals died from prodigiosus septicemia. In spite of the fact that culture tubes showed no growths when inoculated with small quantities of the powder, there were still enough of the living bacilli present to cause fatal infection. This experience fully accords with that of Bertarelli[1] and others, who found that subcutaneous and peritoneal injections of comparatively few living prodigiosus bacilli rapidly cause fatal septicemia in the lower animals.

In the course of two other experiments (rabbits), the work was suddenly cut short by a fatal septicemia which seized the animals, and which proved to be caused by a variety of the colon bacillus.

CONCLUSIONS.

The experiments tried up to the present time clearly indicate that in the animal body, at least, the products of B. prodigiosus exert a marked antagonistic action towards the anthrax bacillus. Such antagonism may even go so far as entirely to protect the animal against fatal anthrax infection. The successful employment, however, of prodigiosus powder in immunization work meets with a serious obstacle at the present time. The powder exerts such a degree of toxic action, when injected, that only very small quantities can be used without serious consequence to the animal. Attempts thus far made to destroy the toxic properties without lessening the protective action have not given the desired results.

The writer is indebted for much valuable help to Professor E. Levy of Strassburg, under whose guidance the work recorded in this paper was begun.

[1] *Centralbl. f. Bakt.*, 1903, 34, pp. 193 and 312.

THE RELATION BETWEEN SERUM RESISTANCE AND VIRULENCE.*

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A number of investigations into the interactions between bacteria and serum in vitro have been reported within the last few years. In these investigations bacteria have been subjected to the action of their own immune serum, of other immune sera, of active normal serum, and of inactive normal serum. Most, if not all, of the experiments previously reported have been upon certain pathogenic forms, especially B. typhosus, B. anthracis, and the cholera vibrio.

Increase in serum resistance together with increased virulence has been observed in B. typhosus by Walker[1] and Steinhardt.[2] Walker secured a markedly increased serum resistance and an increase in virulence for both rabbits and guinea-pigs by passage through normal rabbit blood. Steinhardt observed increased serum resistance in only one of four series passed through normal horse serum. In this one series of 11 passages the virulence was increased sevenfold. In another series of 43 passages, virulence was increased twofold, but there was no increase in serum resistance. Serum resistance did not develop in the other two series, no virulence tests being made. Shaw[3] found a fourfold increase of virulence for B. anthracis after five passages through normal human serum. While he made no experiments to determine the serum resistance, he concluded from the character of the growths observed during several passages, that the organism underwent a gradual immunization against the action of the serum.

Increased serum resistance of B. anthracis to normal rabbit serum without increase in virulence has been reported by Sacharoff.[4] Cohn[5] obtained increased resistance in B. typhosus by

* Received for publication September 18, 1905.

1 *Brit. Med. Jour.*, 1902, 2, p. 1199.

2 *Jour. Med. Res.*, 1905, 13, p. 409.

3 *Brit. Med. Jour.*, 1903, 1, p. 1074.

4 *Centralb. f. Bakt.*, 1904, 37, p. 411.

5 *Ztschr. f. Hyg.*, 1903, 45, p. 61.

passage through active rabbit serum, but no resistance when passed through inactivated serum. The same results were obtained by Trommsdorf[1] with B. typhosus and with the cholera vibrio. Neither of the latter investigators tested for virulence.

Increased virulence of cholera vibrios after passage through normal horse serum and normal guinea-pig serum has been reported by Hamburger.[2]

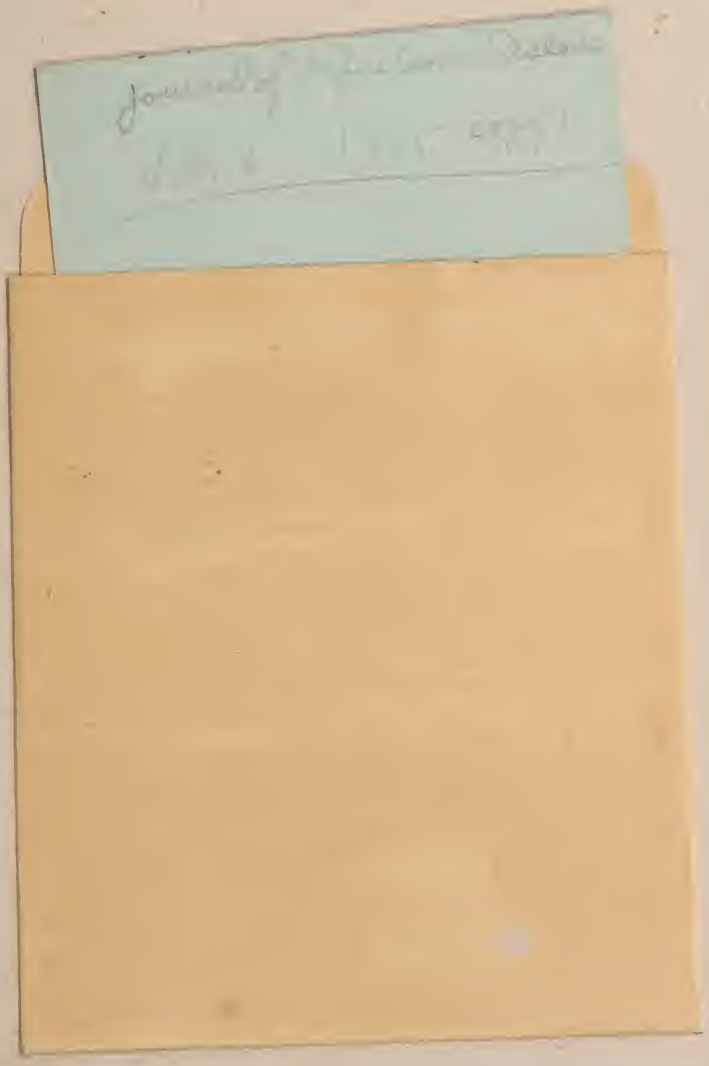
On the whole, examination of the literature upon experiments with normal bactericidal serum shows that the results have been rather irregular. Increase in serum resistance is noted in most instances, but is not always accompanied by increase in virulence.

In the experiments here reported, three relatively nonpathogenic organisms were used; namely, B. prodigiosus, B. proteus vulgaris, and B. fluorescens nonliquefaciens. All were taken from stock cultures that had been on artificial media in this laboratory for several years, and were found to exhibit typical cultural characteristics.

The method of serum culture was similar to that of Ainley Walker.[3] The cultures were made upon slant agar, so slanted as to give approximately the same area to each surface. After 24 hours an emulsion of the growth was made in five c.c. of sterile normal salt solution. Dilutions ranging from one in one thousand to one in ten million were made of this emulsion, and two c.c. of each dilution placed in a small serum tube. Two c.c. of fresh normal rabbit serum were added to each tube, the mixture incubated for three hours, and then plated. At the end of 24 hours a transfer from plate to slant agar was made from the highest dilution which showed growth, thus securing those organisms which had been subjected to the strongest action of the serum. After several passages, to obtain a more concentrated action of the serum, three c.c. of serum were added to each two c.c. of suspension, and later four c.c. A control or parallel series for each organism was run on slant agar.

First series.—This series consisted of 10 passages by the method described above. After three daily transfers on agar,

[1] *Archiv f. Hyg.*, 1900-01, 39, p. 31.

[2] *Wien. klin. Wchnschr.*, 1903, 16, p. 97.

[3] *Brit. Med. Jour.*, 1902, 2, p. 1199.